Imaging Human Atherosclerosis with \(^{99m}\)Tc-labeled Low Density Lipoproteins

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The feasibility of localizing human atherosclerotic plaques by gamma scintillation camera external imaging with technetium-99m-labeled low density lipoproteins (\(^{99m}\)Tc-LDL) was tested in 17 patients who had atherosclerosis. Imaging demonstrated focal accumulation of radiolabel consistent with \(^{99m}\)Tc-LDL sequestration by plaques in the carotid, iliac, or femoral vessels of four patients 8 to 21 hours after intravenous injection of the radiopharmaceutical. Focal accumulation of \(^{99m}\)Tc-LDL also appeared in the location of coronary lesions in four patients, but this accumulation could not be distinguished with certainty from residual blood pool radioactivity. When carotid endarterectomy specimens from six patients who received \(^{99m}\)Tc-LDL 1 day before endarterectomy were examined, the specimens had focal accumulations of radiolabel, with two to four times greater radioactivity in some regions of each specimen than in others; this occurred whether or not the lesions were detected on the gamma camera images. Lesion composition may have determined whether accumulation was quantitatively sufficient to produce an external image. Histologically, the imaged carotid specimen had abundant foam cells and macrophages and poorly organized intramural blood consistent with a plaque hemorrhage; in contrast, nonimaged endarterectomy specimens were mature, fibrocalcific plaques. We conclude that: 1) \(^{99m}\)Tc-LDL did accumulate in human atherosclerotic plaques; 2) In some patients, the accumulation of \(^{99m}\)Tc-LDL was sufficient for detection by gamma camera imaging; 3) the amount of LDL that accumulated appeared to depend on lesion composition; and 4) the design of new radiopharmaceuticals with reduced residual blood pool activity relative to plaque accumulation should lead to improved external imaging of atherosclerosis.

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radiolabel to permit external imaging with the gamma camera, and in carotid endarterectomy specimens from six of the subjects, to determine the distribution of radiolabel within atherosclerotic lesions and correlate tissue composition with the ability to obtain an external image.

Methods

Patients

Seventeen patients were recruited from the Arteriosclerosis Center's outpatient clinic and from among the patients of physicians at the Massachusetts General and New England Deaconess Hospitals. Signed statements of consent were obtained from each patient after the nature of the study had been fully explained. Relevant data for each patient are outlined in Table 1. Six patients (Nos. 3, 4, 7, 8, 10, and 14) underwent carotid endarterectomy after imaging, and their surgical specimens were available for measurement of radioactivity and histologic study. Seven patients (Nos. 1, 2, 3, 7, 10, 11, and 12) were on lipid-lowering medications, with varying degrees of success.

Preparation of Radiolabeled Low Density Lipoprotein

On Day 1, 100 ml of blood was drawn from an antecubital vein under sterile precautions and the patient's LDL was isolated on Day 1 and Day 2 by sequential ultracentrifugation as described previously. On Day 3, the LDL was radiolabeled with $^{99m}$Tc under sterile conditions, as follows. Two to 6 mg of LDL (at a density of 1.050 g/ml) were mixed, and to them was added 10 mg of sodium dithionite dissolved immediately before use in 0.1 ml of 0.5 M glycine buffer (pH 9.8). The total volume of the reaction mixture was about 1.1 ml. This mixture was allowed to stand at room temperature for 30 minutes (20 minutes for Patients 1 and 7); then it was chromatographed on a sterile 2.5 x 10 cm disposable Sephadex G-50 column (Pharmacia Incorporated, Piscataway, NJ) which was pre-equilibrated with sterile 0.15 M sodium chloride brought to pH 8 with sterile sodium bicarbonate buffer. After a volume equal to that of the reaction mixture was allowed to flow through the column, 7.5 ml of the saline/bicarbonate buffer was used to elute the column. The first 4 ml of eluate was discarded. The next 3.5 ml, which contained the $^{99m}$Tc-LDL, was collected in a sterile 30 ml technetium elution vial (E.I. du Pont de Nemours & Company, North Billerica, MA). The reactants were mixed, and to them was added 10 mg of sodium dithionite dissolved immediately before use in 0.1 ml of 0.5 M glycine buffer (pH 9.8). The total volume of the reaction mixture was about 1.1 ml. This mixture was allowed to stand at room temperature for 30 minutes (20 minutes for Patients 1 and 7); then it was chromatographed on a sterile 2.5 x 10 cm disposable Sephadex G-50 column (Pharmacia Incorporated, Piscataway, NJ) which was pre-equilibrated with sterile 0.15 M sodium chloride brought to pH 8 with sterile sodium bicarbonate buffer. After a volume equal to that of the reaction mixture was allowed to flow through the column, 7.5 ml of the saline/bicarbonate buffer was used to elute the column. The first 4 ml of eluate was discarded. The next 3.5 ml, which contained the $^{99m}$Tc-LDL, was collected in a sterile 30 ml technetium elution vial (E.I. du Pont de Nemours & Company). The term $^{99m}$Tc-LDL, as used throughout this report, refers to the 3.5 ml of eluate obtained from the Sephadex G-50 column. It had been previously shown by ultracentrifugation and paper electrophoresis that this preparation consisted predominantly of $^{99m}$Tc tightly bound to LDL along with a much smaller, variable amount of macromolecular inorganic technetium species. In the present study, each preparation was filtered through a 0.22um filter just before use, and a small aliquot was analyzed by paper electrophoresis as described previously. In each case, the major peak was that of $^{99m}$Tc-LDL, similar to the published example, with a variable amount of radioactivity which, as reported previously, did not stain with oil red O after paper electrophoresis.

Imaging

The patients were immediately imaged with either a standard or a large field-of-view gamma camera equipped with an all-purpose parallel-hole collimator, and then imaged again one to three times up to 25 hours after injection of 10 to 20 mCi of $^{99m}$Tc-LDL. Anterior cervical and thoracic views, anterior and posterior abdominal views, and anterior views of the pelvis and lower extremities were recorded in all patients; additional views were recorded when appropriate. Images were obtained for up to 10^6 counts for each view, or up to 15 minutes at the later imaging times. All images were reviewed by three nuclear medicine physicians. Only regions of arterial accumulation identified as positive by all three reviewers were called positive.

Specimen Analysis

Six carotid endarterectomy specimens were available for direct examination; specimens of other arteries were not available for analysis.

After gross photography, the carotid endarterectomy specimens were rinsed in normal saline until the rinses were free of blood, and then divided into three to four sections of differing atherosclerotic involvement based on the degree of mural thickening and stenosis. The tissues were blotted lightly before being weighed and were then counted in a gamma counter (Packard 5630, Packard Instrument Corporation, Des Plaines, IL).

After measurement of radioactivity, the sections of each carotid endarterectomy specimen were fixed in 10% formalin, embedded in paraffin, cut in cross-section for light microscopy, and stained with hematoxylin and eosin. A maximum of 2 hours elapsed between the time carotid specimens were removed surgically and the time they were placed in fixative.

To determine the percent of injected $^{99m}$Tc that was excreted in urine, 24-hour urine collections were obtained, measured, and counted in three patients.

Definitions

A positive gamma scintillation camera image was defined as the appearance of focal radiolabel accumulation in the region of a vessel visible above the blood pool that increased in intensity over time relative to blood pool radioactivity and was asymmetric relative to the contralateral vessel.

In carotid endarterectomy specimens, focal accumulation was defined as higher levels of radioactivity in some regions of the specimen and lower levels elsewhere.

The histologic sections of carotid specimens were classified by a semiquantitative assessment of the fraction of each section that had abundant macrophages and foam cells, and/or hematoma or necrosis (Category A) relative to the fraction of the section that was mature fibrous connective tissue, with or without areas of calcification (Category B). The primary distinction was between tissue in which the functional metabolic status and/or the structural organization appeared to be evolving...
and tissue which appeared to be quiescent. Since the regions within the plaques were not sharply delineated, exact morphometric measurements of the specimens could not be made. Tissue in Category A had more than 50% dense inflammatory infiltrate with or without abundant macrophages and foam cells, and/or unorganized or incompletely organized intramural blood or focal necrotic debris. Tissue in Category B was mature fibrous connective tissue, which contained smooth muscle cells and/or fibroblasts, with or without dystrophic calcification. "Intermediate tissue" was sparsely cellular and densely fibrocalcific, with only small regions of macrophages, foam cells, hemorrhage, or necrosis.

**Results**

**Scintigraphy**

The typical appearance of sequential gamma camera images of a patient given $^{99m}$Tc-LDL is illustrated in Figure 1. Immediately after injection of the radiolabel, blood pool radioactivity was evident in several organs. Over a 15-hour period, the cardiac radioactivity decreased, while radioactivity in the liver, intestine, and kidney increased relative to the heart. The shift in relative radioactivity from the heart, which is a blood pool marker, to organs known to metabolize LDL$^{16,17}$ was consistent with the transfer of radioactivity from blood to tissue and indicated that the radiopharmaceutical had the biological characteristics of native LDL$^{16,17}$. Four patients (Table 1, Nos. 1 to 4) accumulated sufficient $^{99m}$Tc-LDL to produce images with the gamma camera in locations where atherosclerotic arterial lesions were known or likely to be present; four patients (Nos. 2, 5, 6, and 9) accumulated radiolabel in areas of the heart anatomically consistent with the location of coronary arteries known or likely to be atherosclerotic, but these areas could not be distinguished with certainty from blood pool radioactivity; ten patients (Nos. 7, 8, and 10 to 17) had no focal arterial accumulation of radiolabel. The doses of $^{99m}$Tc-LDL for each patient and the time parameters of imaging are listed in Table 2.

Arteries with focal accumulation of radiolabel that was consistent with a positive image included the iliac, femoral, and carotid vessels. Patients 1, 2, and 4 had images of the iliac and/or femoral arteries. Patient 1, who had a
<table>
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<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>TC*</th>
<th>TG†</th>
<th>Vascular events or symptoms‡</th>
<th>Time before imaging</th>
<th>Related vascular procedures‡</th>
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<th>Current symptoms</th>
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<th>Carotid specimen§</th>
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<td>224</td>
<td>66</td>
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<td>R femoral embolectomy</td>
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<td>+</td>
<td>Iliofemoral</td>
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<td>6 yrs</td>
<td>-</td>
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<td>3</td>
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<td>M</td>
<td>199</td>
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<td>Coronary bypass</td>
<td>2.5 yrs</td>
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<td>4</td>
<td>72</td>
<td>M</td>
<td>201</td>
<td>95</td>
<td>Femoral bruit</td>
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<td>-</td>
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<tr>
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<td>42</td>
<td>M</td>
<td>146</td>
<td>149</td>
<td>MI</td>
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<td>66</td>
<td>F</td>
<td>294</td>
<td>503</td>
<td>Angina</td>
<td>16 yrs</td>
<td>None</td>
<td>5 days</td>
<td>-</td>
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</table>

* TC = Total cholesterol; TG = Triglycerides

‡ Time in years (yrs), weeks (wks) or months (mos)

§ Carotid = Carotid angioplasty; Femoral = Femoral grafts; Aortoiliac = Aortoiliac graft
Table 1. (Continued)

<table>
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<th>Patient no.</th>
<th>Age (yrs)</th>
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<th>Time before imaging</th>
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<th>Time before imaging</th>
<th>Current symp-toms</th>
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<td>M</td>
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<td>196</td>
<td>L carotid stenosis</td>
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<td>5</td>
<td>M</td>
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<td>R carotid stenosis</td>
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<td>—</td>
<td>—</td>
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<td>Coronary insufficiency</td>
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<td>—</td>
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<td>M</td>
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<td>Coronary insufficiency</td>
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<td>—</td>
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</tbody>
</table>

*TC = total plasma cholesterol (mg/dl); †TG = plasma triglycerides (mg/dl); ‡L = left, R = right, MI = myocardial infarction, TIA = transient ischemic attack, t-PA = tissue plasminogen activator; §Carotid specimen = carotid endarterectomy specimen obtained for counting of radioactivity and histological examination.

10-year history of bilateral claudication, had asymmetrical accumulation of radiolabel at several points in the iliofemoral system on the 21-hour images (Figure 2). Patient 2 had a 10-year history of left calf claudication and had left calf claudication at the time of imaging; his 6-hour and 21-hour images showed focal, asymmetric, accumulation of radiolabel in the region of the left femoral artery. Patient 4 had a history of generalized atherosclerosis including recent carotid artery symptoms; although his carotid circulation did not image, his right femoral artery showed focal accumulation of radiolabel at 16 hours.

In addition to the femoral disease described above, Patient 2 also had had a myocardial infarction 10 years before his imaging study. At the time of imaging, he denied cardiac symptoms, but his physical examination and electrocardiogram were consistent with severe coronary artery disease. The 8-hour image showed focal, linear accumulation of radiolabel in an area of the heart that was anatomically consistent with the left anterior descending (LAD) coronary artery; however, the accumulation could not be unequivocally distinguished from blood pool radioactivity. The same problem of interpretation occurred in patients 6 and 9. In Patient 6, focal accumulation in the area of the LAD was evident in the 14-hour and the 22-hour images; angioplasty of the patient's LAD artery had been performed 4.5 years before the imaging study. One month before the study, an angiogram showed diffuse atherosclerosis of the LAD coronary artery and tight stenosis of the circumflex artery on which angioplasty was performed; no focal accumulation of radiolabel was evident in the area of the circumflex artery in the imaging study. Patient 9 developed exertional chest pain 6 months before his imaging study and underwent coronary angioplasty of the LAD artery 6 weeks before imaging. In the 2-hour and 16-hour images, there was focal accumulation of radiolabel in the area of the LAD artery, which intensified over time, but could not clearly be distinguished from blood pool radioactivity. Uncertainty about a coronary image also occurred with Patient 5, who had an occluded right coronary artery recanalized by intracoronary injection of tissue plasminogen activator 1 week before imaging. Focal accumulation of radiolabel anatomically consistent with the location of the proximal right coronary artery was visible on the 16-hour image but could not be distinguished with certainty from blood pool radioactivity.

Patient 3 had a 9-hour image (Figures 3A and 3B) which showed asymmetrical focal accumulation of radiolabel in the right internal carotid artery. An angiogram performed before right carotid endarterectomy (Figure 3C) showed tight stenosis of the right internal, and moderate stenosis of the left internal, carotid arteries. These lesions were associated with the recent onset of a prominent high-pitched right carotid bruit and no bruit on the left. At surgery, a brown-stained tightly stenotic right carotid lesion was removed, which appeared on gross examination to have an organized, relatively recent intramural hemorrhage (Figure 3D).

**Histology**

Histologic examination of arterial specimens from the six carotid endarterectomy patients made possible a

*Later images could not be obtained because of surgical scheduling.*
Figure 2. Anterior scintigram of Patient 1 at 21 hours after intravenous injection of 18.6 mCi of $^{99m}$Tc-LDL. The field of view included the area between the umbilicus and mid-thigh. In addition to accumulation in bowel and genitalia, the distribution of radiolabel in the iliac and femoral vessels was asymmetrically irregular (arrows).

correlation between lesion composition as assessed by the light microscopic appearance and the ability to image. The area of tight stenosis in the imageable lesion of Patient 3 was largely composed of poorly organized intramural hematoma with abundant foam cells and macrophages (Figure. 3E). In contrast, the other five carotid specimens, which did not image, were more fibrocalcific than cellular, hemorrhagic, or necrotic. For example, the right carotid artery of Patient 7, which did not image, appeared tightly stenosed on angiogram (Figure 4A). At surgery, a small, stenotic lesion was removed (Figure 4B) which too small and hard to be cut into sections. The specimen was almost entirely fibrous tissue, with focal calcification and little cellularity (Figure 4C). The lesions in Patients 4, 8, and 14 were also predominantly fibrous, while the lesion in Patient 10 was intermediate with some cellular-hemorrhagic areas and other areas which were fibrotic.

Focal Radioactivity of Carotid Specimens

Although the carotid lesions of five carotid endarterectomy patients (Nos. 4, 7, 8, 10, and 14) did not accumulate enough radiolabel to be visible above the blood pool on external imaging, the five carotid specimens that could be sectioned (including that of Patient 3, which did image) showed focal accumulation of radioactivity, with up to two
to four times more radioactivity in the centers of plaques than elsewhere in the specimens (Table 3). Because of the complexity of the studies, it was not feasible to obtain serial blood samples to measure the average plasma radioactivity, which is necessary to make quantitative comparisons of LDL accumulation among the specimens. In addition, surgical scheduling precluded the possibility of obtaining all the carotid specimens at the same time after injection of $^{99m}$Tc-LDL. However, even with these limitations in mind, it was clear that the difference in radiolabel accumulation by Patient 3's specimen, which imaged, and the other carotid specimens was marked. The anterior and posterior portions from the center of Patient 3's plaque accumulated $20 \times 10^{-4}$% and $18 \times 10^{-4}$%, respectively, of the injected dose per gram. In contrast, the two center portions of the fibrotic lesion of Patient 4 accumulated less than $1 \times 10^{-4}$% of the injected dose per gram. Radiolabel accumulation by other fibrotic lesions was similar. The histologically intermediate lesion of Patient 10 accumulated no more than $2 \times 10^{-4}$% of the injected dose per gram in any portion of the specimen.

Urine Radioactivity

Cumulative urinary excretion of $^{99m}$Tc-LDL was measured in three patients. Over a 24-hour period from the
Figure 3. Imaging studies and pathology of carotid stenosis in Patient 3. A. External image 9 hours after injection of 13.1 mCi of $^{99m}$Tc-LDL showing focal, asymmetrical accumulation of radiolabel in the right distal common carotid artery at the carotid bifurcation and the proximal internal carotid artery (arrows). B. Sketch of A. C. Right carotid angiogram showing extensive stenosis (large arrow) and mural irregularities (small arrows) involving the distal common and proximal internal carotid arteries. This corresponded to the region of accumulation of $^{99m}$Tc-LDL in the gamma camera image. The left carotid angiogram showed only a minor degree of internal carotid stenosis. D. Photograph of bisected right carotid endarterectomy specimen showing the tight stenosis (arrow) and the long underlying, poorly organized deep hematoma covered by a fibrous cap. E. Photomicrograph of lesion showing numerous foam cells and macrophage giant cells adjacent to a poorly organized intramural hematoma. Hematoxylin and eosin stain. × 200
Figure 4. Angiogram and pathology of carotid stenosis in Patient 7. This patient had no focal vascular accumulation of radionuclide. A. Right carotid angiogram showing a tight stenosis at the origin of the internal carotid artery (arrow). B. Photograph of bisected right carotid endarterectomy specimen. The stenosis (arrow) was apparent, although the calcific composition of the lesion was not visible in the photograph. C. Photomicrograph of lesion. There is a mature, fibrous plaque, with rare macrophages and foam cells. Hematoxylin and eosin stain. ×200

Discussion

The goal of this study was to demonstrate the feasibility of detecting atherosclerotic plaques by their intramural accumulation of a radiopharmaceutical, rather than by their space-occupying characteristics, as is current practice. The approach is based on the noninvasive technique of gamma camera scintigraphy and takes advantage of previous observations that radiolabeled LDL is rapidly focally sequestered in abnormal arterial wall. The earlier observations were confirmed and extended in the present study, which showed that some human atherosclerotic lesions in the carotid, iliac, and femoral circulations could be imaged externally with the gamma scintillation camera after injection of 99mTc-LDL. Based on the data obtained from carotid endarterectomy specimens, lesion composition may have determined whether 99mTc-LDL accumulation was quantitatively sufficient to produce an external image. The carotid plaque that did image was made up of tissue that could be described as being in a state of active evolution; it had abundant foam cells and macrophages as well as an area of poorly organized intramural hemorrhage. The total accumulation of radionuclide by this specimen was far greater than that of any other specimen. Conversely, the five fibrocalcific carotid plaques that did not image were made up of tissue which could be described as quiescent, or "burned-out." However, regardless of whether they imaged, all five specimens that could be subdivided had greater radioactivity in the centers of plaques and less radioactivity elsewhere. These findings support the concept that the atherosclerotic process pro-
In addition to the specific tissue characteristics of plaques, a major determinant of the ability to image with the gamma camera is the relationship between the radioactivity in the lesion and that in the blood pool, known as the target-to-background ratio. In this study, the target-to-background ratio obtained with $^{99m}$Tc-LDL was clearly not optimal, perhaps because the biological half-life of LDL in the plasma ranges from 2 to 6 days, while the physical half-life of $^{99m}$Tc is only 6 hours. This led to uncertainty in the interpretation of focal cardiac radiolabel accumulation in four patients, and in light of the focal accumulation of radiolabel measured ex vivo in the carotid endarterectomy specimens, could well have led to negative imaging studies in the presence of active disease. We are now investigating a variety of ways to improve the target-to-background ratio of the imaging radiopharmaceutical. Although a method of attaching to LDL an imaging isotope with a half-life longer than the 6 hours of $^{99m}$Tc is not yet available, this is one possible approach. However, $^{99m}$Tc does have the advantage of being readily available and inexpensive, and it appears to be partially trapped intracellularly, as indicated by the 24-hour urinary excretion of radiolabel, which ranged only from 4% to 12%; this finding, in contrast to results obtained with $^{131}$I-LDL, where 20% to 50% of the isotope appeared in the urine in 24 hours, was consistent with animal data obtained previously. Whatever radiopharmaceuticals prove to be optimal, the present study demonstrates that external imaging of human atherosclerosis is feasible and may aid in differentiating quiescent from actively evolving plaques.

### Acknowledgments

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