Noninvasive Imaging of $^{99m}$Technetium-labeled Low Density Lipoprotein Uptake by Tendon Xanthomas in Hypercholesterolemic Patients

Henry N. Ginsberg, Stanley J. Goldsmith, and Shankar Vallabhajosula

Technetium-labeled low density lipoproteins (Tc-LDL) appear to be useful for describing LDL biodistribution in normal and dyslipidemic subjects. We injected $^{99m}$Tc-LDL into subjects with large tendon xanthomas secondary to homozygous familial hypercholesterolemia or sitosterolemia. Rapid (4 hours) accumulation of $^{99m}$Tc activity in xanthomas was observed, and this accumulation increased over a 24-hour period. No comparable accumulations of $^{99m}$Tc activity were noted in normal subjects or in a subject with heterozygous familial hypercholesterolemia who had very small tendon xanthomas. These findings support previous biopsy data indicating active uptake of LDL by macrophages within xanthoma and suggest that $^{99m}$Tc-LDL imaging of xanthomas may be useful in studies of the effects of diet and drugs on the accumulation of lipoproteins by atherosclerotic plaques. (Arteriosclerosis 10:256-262, March/April 1990)

Xanthomas are accumulations of lipids in the skin and tendons of individuals with various forms of hyperlipidemia. The major lipids in xanthomas are usually esterified and nonesterified cholesterol. Pathological studies have demonstrated that "foam cells," or lipid-laden macrophages, are the major cell type found in most xanthomas, although lipid-filled fibroblasts and dermal cells have also been described. The mechanisms underlying the accumulation of cholesterol in these cells are unclear. Scott and Winterbourn demonstrated accumulation of intravenously injected radiiodinated low density lipoproteins (LDL) in biopsies of xanthomatous tissues from hypercholesterolemic subjects and concluded that the accumulation could not be explained simply on the basis of the vascularity of the xanthoma.

Technetium $^{99m}$-labeled LDL (Tc-LDL) was first shown by Lees et al. to be useful as an agent to image LDL distribution in vivo. Labeling of LDL with Tc does not appear to alter its native, biological activity as assessed by in vivo kinetic studies and by biodistribution data. We further demonstrated that Tc-LDL, unlike radiiodinated LDL, acted in a manner similar to tyramine cellulose-labeled LDL and was accumulated almost quantitatively after uptake into tissues. This characteristic of Tc-LDL enabled us to demonstrate changes in tissue distribution of Tc-LDL in hypercholesterolemic rabbits compared to normal rabbits and abnormal accumulation of LDL in spleen and bone marrow of patients with myeloproliferative disorders.

Our finding, that accumulation of LDL in the marrow of long bones in subjects with myeloproliferative disorders correlated with the presence of macrophages at those sites, suggested that Tc-LDL might be useful as an agent to image xanthomas in subjects with hypercholesterolemia. In this report, we present evidence that Tc-LDL is actively taken up by xanthomas and suggest, therefore, that external imaging of xanthomas might be a useful, noninvasive approach to assess the metabolic state of atheromatous lesions.

Methods

Subjects

One normal subject and four hypercholesterolemic subjects were studied. Their clinical characteristics are presented in Table 1. In the hypercholesterolemic group, two individuals were homozygous for familial hypercholesterolemia (HFH), one subject was heterozygous for familial hypercholesterolemia (FH), and one subject had sitosterolemia. The two HFH patients were siblings who had large tendonous, tuberous, and planar xanthomas. They had no clinical evidence of coronary artery disease themselves. Studies of their fibroblast LDL receptors and of the LDL receptor gene, performed by Helen Hobbs in the laboratory of Joseph Goldstein and Michael Brown, revealed that these patients were homozygous for a previously reported mutation that results in a defect in the internalization of the LDL receptor after binding of LDL. The $^{99m}$Tc-LDL scans in the two HFH subjects were obtained while they were untreated.

The heterozygous FH patient had small tendon xanthomas on her proximal interphalangeal joints, on both
Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>TC</th>
<th>TG</th>
<th>HDLC</th>
<th>LDLc</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFH 1</td>
<td>25</td>
<td>M</td>
<td>600</td>
<td>120</td>
<td>34</td>
<td>542</td>
<td>None</td>
</tr>
<tr>
<td>HFH 2</td>
<td>16</td>
<td>F</td>
<td>675</td>
<td>80</td>
<td>32</td>
<td>627</td>
<td>None</td>
</tr>
<tr>
<td>Hetero FH</td>
<td>45</td>
<td>F</td>
<td>350</td>
<td>120</td>
<td>75</td>
<td>251</td>
<td>c,n,l</td>
</tr>
<tr>
<td>Sitostolemia</td>
<td>57</td>
<td>F</td>
<td>280</td>
<td>125</td>
<td>40</td>
<td>215</td>
<td>c</td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>M</td>
<td>138</td>
<td>90</td>
<td>40</td>
<td>80</td>
<td>None</td>
</tr>
</tbody>
</table>

TC=total cholesterol, TG=total triglycerides, HDLC=high density lipoprotein cholesterol, LDLc=low density lipoprotein cholesterol, HFH=homozygous familial hypercholesterolemia, FH=familial hypercholesterolemia.
*Treatment at the time of study: c=cholestyramine, n=niacin, l=lovastatin.
†Plasma lipid concentrations were obtained during treatment in the heterozygous FH and the sitosterolemic subjects.

Preparation of Radiopharmaceutical

LDL was isolated from each subject’s plasma at the density interval 1.019 to 1.063 g/ml by sequential ultracentrifugation and sterile techniques. The LDL was dialyzed against several liters of normal saline, pH 7.2, containing 0.1 mg/ml of ethylenediaminetetraacetic acid, and then labeled with Tc-99m by reductive coupling. Briefly, 1 to 2 mg of LDL (0.5 ml) was mixed with 50 mCi of Tc-99m pertechnetate (0.5 ml) and 0.1 ml of glycine buffer containing 10 mg of sodium dithionite, and the mixture was incubated for 30 minutes. The Tc-99m-LDL was separated from free Tc-99m by Sephadex G-25 chromatography. The gel-filtered Tc-99m-LDL was sterilized by passage through a 0.22 µm Millipore filter before injection into subjects. The in vivo imaging studies were performed after the subjects had fasted overnight. Ten mCi of Tc-99m-LDL was injected intravenously within 30 minutes of radiolabeling.

 Imaging Studies

The biodistribution of Tc-99m-LDL was imaged with a gamma camera (GE-400T) fitted with a low-energy, all-purpose, parallel-hole collimator. Anterior and posterior views of the total body were obtained at 30 minutes and at 1, 4, and 24 hours after the injection of Tc-99m-LDL. The images were stored in a 128x128 matrix of an imaging computer (Siemens-Microdelta). The scans were read by an experienced nuclear medicine physician (S.J. Goldsmith).

Results

The normal subject was a 23-year-old man with normal plasma concentrations of total, LDL, and high density lipoprotein cholesterol. The biodistribution of Tc-99m-LDL in this subject was essentially identical to that of the three other normal subjects reported previously. The total body distribution of Tc-99m-LDL in the normal subject is shown in Figure 1A. Immediately after injection, Tc-99m activity was seen mainly in major blood vessels, liver, and kidney. In HFH, however, there was an increased accumulation of Tc-99m activity over both knees (arrowhead), corresponding to an area of xanthomas.

Figure 1. The distribution of technetium-labeled low density lipoproteins (Tc-LDL) in a normal subject (A) and in a patient (B) who is homozygous for familial hypercholesterolemia (HFH 1). Whole-body Images were obtained at 4 hours after the Intravenous Injection of Tc-LDL In both subjects, Tc-99m activity was seen mainly in major blood vessels, liver, and kidney. In HFH, however, there was an increased accumulation of Tc-99m activity over both knees (arrowhead), corresponding to an area of xanthomas.
Figure 2. Technetium-labeled low density lipoprotein (Tc-LDL) accumulation in the xanthomas of the familial hypercholesterolemic patient depicted in Figure 1. A. Photograph of the xanthoma involving each knee. B. A close-up gamma camera image of Tc-99m accumulation of these sites 4 hours after injection of Tc-LDL.

was also evident at this time. Over the 24 hours of study, activity in the major blood vessels declined continuously while that over the organs (liver, kidney, and spleen) increased. Based on these observations, we chose to present 4- and 24-hour images for the purposes of this study.

Figure 1B depicts the total body distribution of Tc-LDL in HFH 1. In general, LDL biodistribution in this subject was comparable to that obtained in the study of the normal subject (Figure 1A). Increased Tc-99m activity could be seen, however, over both knees in HFH 1. This difference, which was accompanied by increased uptake over his elbows and Achilles’ tendons (see below), persisted through the 24-hour image. During this same period of time, Tc-99m activity over the liver increased, while that in the major vessels declined.

Closer views of the knees of HFH 1 can be seen in Figure 2. Figure 2A shows the xanthoma involving each of his knees. These were yellow-orange in color and had a firm consistency. Figure 2B is an image of the Tc-99m activity over this region. It is clear that there was significant accumulation of activity in this area.

HFH 2 also had large xanthomas over her knees and her Achilles’ tendons (Figure 3), and these areas accumulated significant Tc-99m activity by 4 hours after injection of Tc-LDL (Figure 4A). A scan of the heterozygous FH subject (Figure 4B), who had small, hard elevations over both pretibial tuberosities and some minor thickening over her Achilles’ tendons, did not reveal significant focal uptake of Tc-99m activity, although there was a suggestion of accumulated activity in the area of her Achilles’ and plantar tendons.

The patient with sitosterolemia had very large tuberous xanthomas over her elbows and her hands, and these areas demonstrated increased activity after injection of Tc-LDL (Figures 5A and 5B). She had similar findings over other areas of xanthomatous involvement such as her knees and feet (data not shown). No such accumulations of Tc-99m activity were seen in any of these areas in the normal subject.

Because of the suggestion in our previous work7 that Tc-LDL acts as a trapped ligand after LDL uptake into tissues, urine was collected over the 24-hour period of study in the two subjects with HFH. In these two patients, 8% and 11% of the injected Tc-99m activity was excreted in the urine during that period of time.

Discussion
There is little doubt that the bulk of cholesterol, which is predominantly cholesteryl ester, found in arterial ath-
Figure 3. Photographs demonstrate the xanthomas over the knees and the Achilles’ areas in the second homozygous familial hypercholesterolemic patient (HFH 2).

erosclerotic lesions and in xanthomatous deposits derives from plasma cholesterol, particularly LDL cholesterol. Lipoproteins with densities less than 1.063 g/ml have been isolated from both atherosclerotic and normal aortic tissue, and apolipoprotein B has been identified in arterial lesions. Recently, evidence that “modified” LDL accumulates in the subintimal space has also been presented.

Studies by Armstrong et al. demonstrated increased rates of cholesterol esterification in xanthomas isolated from the skin of hypercholesterolemic rabbits. Scott and Winterbourn injected radiiodinated LDL into hypercholesterolemic rabbits with large xanthomas and demonstrated accumulation of labeled LDL (or LDL fragments) in biopsies of those lesions. Those authors indicated that the ratio of tissue to plasma radioactivity in the xanthomas supported the hypothesis of active uptake and trapping of lipoprotein cholesterol by the lesions. All of these data are consistent with our demonstration of active uptake and accumulation of circulating Tc-LDL by xanthomas in patients with HFH and sitosterolemia.

The mechanism whereby LDL is taken up by macrophages in xanthoma has not been entirely clarified. In the study by Scott and Winterbourn, accumulation of radioiodinated LDL was demonstrated in biopsies of xanthomas obtained at 66 to 72 hours after injection of tracer. In the present study, increased Tc-99m activity was observed by 4 hours after injection of Tc-LDL. The rapidity of accumulation in our studies suggests a high-affinity, receptor-mediated pathway for LDL uptake. Macrophages do not appear to take up normal LDL at very high rates, although internalization of LDL by the LDL receptor does occur in these cells. In a recent preliminary report, Lees and Lees presented evidence that Tc-LDL interacts with the LDL receptor on cultured human fibroblasts, leading to cell uptake of the ligand. The marked accumulation of Tc-99m activity in the large xanthomas in our two HFH patients, who had almost no active LDL receptors, makes it very unlikely, however, that the LDL receptor pathway played a significant role in Tc-LDL uptake in their lesions. This conclusion is supported further by the lack of significant, focal accumulations of Tc-99m in the heterozygous FH subject, who would have had about 50% normal LDL binding and uptake. The lack of xanthoma imaging in the heterozygous FH patient suggests that the number and/or activity of the macrophages in the xanthoma is rate-limiting for Tc-99m accumulation. This hypothesis is consistent with the clear images seen in the patient with sitosterolemia who had plasma LDL levels similar to those of the subject with heterozygous FH, but who had much larger xanthomas than the latter individual. Finally, the accumulation of Tc-99m activity in the xanthomas of the HFH patients also argues against a mechanism whereby our Tc-labeled LDL tracer might have been recognized as β-very low density lipoprotein, which can be efficiently taken up and degraded via the LDL receptor pathway in human monocyte-macrophages.

Recent studies have indicated that LDL modified by lipid peroxidation can be internalized by macrophages via high-affinity scavenger receptors. Our prior results, as well as those of Lees et al. demonstrating normal electrophoretic mobility and normal in vivo plasma kinetics of Tc-LDL, suggest that preparation of the tracer does not create a more negatively charged LDL species. In addition, it is very likely that any significant physical-chemical modification of LDL resulting from Tc-99m labeling would be associated with very rapid removal of the tracer from plasma and significant uptake of Tc-LDL by the spleen and bone marrow of these subjects. We have obtained just such plasma kinetic and tissue biodistribution data after injection of malondialdehyde-modified LDL labeled with Tc-99m into rabbits (Shankar Vallabhajosula and Henry Ginsberg, unpublished data). The lack of increased accumulation of Tc-99m activity in the spleen and bone marrow of the hypercholesterolemic subjects presented in this report in-
Figure 4. The gamma camera image over the knees and Achilles' areas of the homozygous familial hypercholesterolemic patient depicted in Figure 3 4 hours after injection of technetium-labeled low density lipoprotein (\textsuperscript{99m}Tc-LDL) (A) demonstrates clearly that \textsuperscript{99m}Tc accumulates in these areas. B. This panel is the gamma camera image of \textsuperscript{99m}Tc-LDL distribution in a patient with heterozygous familial hypercholesterolemia who had very small xanthomas over the tibial tuberosity on each leg and some mild thickening of her Achilles' tendons. There were no distinct, focal accumulations of \textsuperscript{99m}Tc activity over the knees or Achilles' tendons in the heterozygous patient although there was a suggestion that increased activity was present in the area of the Achilles' and plantar tendons.

Indicates that the accumulation of tracer by their xanthomas is the result of mechanisms occurring locally at the site of those lesions. It is possible that Tc-LDL is oxidized when exposed to large numbers of tissue macrophages in xanthoma and that the modified LDL is then rapidly internalized by those cells. We recently reported increased uptake of \textsuperscript{99m}Tc-LDL by splenic and bone marrow macrophages in patients with myeloproliferative disorders.\textsuperscript{a} An increased capacity for lipid peroxidation has been demonstrated in patients with myeloproliferative disorders, who also have increased numbers of tissue macrophages.

Our demonstration that Tc-99m activity accumulated over xanthomatous lesions during the 24 hours of study supports our initial suggestion\textsuperscript{7} that \textsuperscript{99m}Tc-LDL acts as a trapped ligand after internalization by cells. The low rate of urinary excretion of Tc-99m demonstrated in this study further supports this view, as does recent data from Lees et al.\textsuperscript{27} Preliminary data reported by Lees and Lees\textsuperscript{21} from studies with cultured fibroblasts also support the conclusion that Tc-99m is trapped within cells after uptake of LDL. This characteristic of \textsuperscript{99m}Tc-LDL increases its potential as a tracer for imaging vascular atherosclerosis.

Correlations between the presence of planar and tendon xanthomas and the presence of atherosclerotic cardiovascular disease\textsuperscript{28} have generated interest in xanthomas as a model of lipid deposition in the vessel wall. Regression of
xanthomas has been demonstrated in hypercholesterolemic patients receiving aggressive lipid-lowering therapy and in FH patients entered in plasmapheresis programs to lower LDL cholesterol concentrations. These results are indicative of the presence of a labile pool of cholesterol within xanthomas that is responsive to the circulating levels of plasma cholesterol. These data are paralleled by results demonstrating that atherosomatous lesions contain some cholesterol in a labile pool from which efflux can occur. The use of trapped ligands that can be imaged externally, such as Tc-LDL, offers the possibility of utilizing xanthomas as models for investigations of the effects of diet and drug interventions on the accumulation of lipoproteins by atherosclerotic plaques.

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**References**


Figure 5. Technetium-labeled low density lipoprotein (99mTc-LDL) accumulation in the patient with sitosterolemia who had very large tuberous xanthoma over her elbows and hands. Gamma camera images obtained at 24 hours after the injection of 99mTc-LDL clearly showed abnormally increased accumulation of Tc-99m in her elbows (A) and hands (B) corresponding to the areas where xanthomas were present.

Index Terms: nuclear imaging • technetium-99m • low density lipoproteins • tendon xanthomas
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