Indium-111-Labeled LDL: A Potential Agent for Imaging Atherosclerotic Disease and Lipoprotein Biodistribution

Joel M. Rosen, S. Patrick Butler, George E. Meinken, Theodore S. T. Wang, Rajasekhar Ramakrishnan, Suresh C. Srivastava, Philip O. Alderson, and Henry N. Ginsberg

Departments of Radiology (Nuclear Medicine) and Medicine, Columbia University College of Physicians and Surgeons, New York, New York, and Medical Department, Brookhaven National Laboratory, Upton, New York

Radiolabeling of low-density lipoprotein (LDL) and external imaging with a gamma camera would offer a means of taking advantage of the metabolic activity of developing atherosclerotic lesions in order to noninvasively detect and determine the extent of atherosclerotic cardiovascular disease. Indium-111-\(^{111}\text{In}\) labeled LDL was prepared and its purity demonstrated by agarose electrophoresis and ultracentrifugation. In vitro studies with cultured human fibroblasts demonstrated significant inhibition of iodine-125-\(^{125}\text{I}\) LDL binding to LDL receptors by \(^{111}\text{In}\)-LDL, although this was less than the inhibition produced by unlabeled LDL. Adrenal gland uptake of \(^{111}\text{In}\)-LDL by hypercholesterolemic rabbits was reduced by 86% compared to the level of uptake observed in normal rabbits. These results were compatible with downregulation of adrenal LDL receptors in the hypercholesterolemic rabbits. Uptake of \(^{111}\text{In}\)-LDL in the atherosclerotic proximal aorta of hypercholesterolemic rabbits was 2.5 times higher than in normal rabbits. These results suggest that \(^{111}\text{In}\)-LDL has the potential to be a useful agent for external imaging of atherosclerotic lesions and lipoprotein biodistribution.


Radioiodinated low-density lipoprotein (LDL) has been found to behave like native LDL and has been used successfully for many years in animal studies of LDL accumulation in vascular tissues (2–4). More recently, investigations with LDL labeled with iodine-125 (\(^{125}\text{I}\)) demonstrated the feasibility of using this technique for external imaging in vivo (5). Iodine-125, which emits a very low-energy photon, is, however, a poor label for external imaging. Very low density lipoprotein (VLDL) has been labeled with \(^{123}\text{I}\) for in vivo studies of its metabolism (6), and LDL has been labeled with technetium-99m (\(^{99m}\text{Tc}\)) (7,8). These radiocarbon detectors emit photons that are suitable for imaging, but their relatively short half-lives (\(^{123}\text{I}\): 13 hr, \(^{99m}\text{Tc}: 6\) hr) allow only short-term experiments to be conducted.

Due to the slow plasma clearance of LDL, particularly in hypercholesterolemic individuals (9), experiments of longer duration are necessary for effective imaging of LDL uptake into atherosclerotic lesions. In a recently published study of \(^{99m}\text{Tc}\)-labeled LDL in patients with atherosclerotic disease (10), the authors indicated that some lesions were detected with a gamma camera, but many areas of known disease were not detected because the slow plasma clearance of LDL and the short physical half-life of \(^{99m}\text{Tc}\) resulted in low target-to-background ratios.

We sought to label LDL with indium-111 (\(^{111}\text{In}\)), a gamma emitting isotope with a half-life of 2.83 days, that has been used successfully to label other radiopharmaceuticals (11–13). This isotope is well suited for experiments with labeled lipoproteins because its half-life is sufficiently long to allow imaging of atherosclerotic lesions after blood-pool clearance has occurred and because it emits photons of suitable energies (171 and 245 keV) for efficient imaging with standard gamma cameras. In the current studies, the behavior of \(^{111}\text{In}\)-LDL was tested in vitro, by determining its ability to specifically bind to LDL receptors on cultured fibroblasts, and in vivo, by determining its biodistribution in normal and in hypercholesterolemic rabbits.
METHODS

Preparation of $^{111}$In-LDL

LDL was isolated from human hyperlipidemia plasma by sequential ultracentrifugation (14) and dialyzed overnight against normal saline (pH 7.4) containing 0.1 mg/ml EDTA. Aliquots were taken for determination of protein concentration by the method of Lowry et al. (15). Centricon-30 microconcentrators (Amicon Division, W. R. Grace & Co., Danvers, MA) were used for most buffer changes and washes of LDL during subsequent processing. LDL was added to microconcentrators which had been pretreated with 1% bovine serum albumin in 0.1 N NaHCO₃, and the buffer changed from normal saline (pH 7.4) containing 0.1 mg/ml EDTA to 0.1 N NaHCO₃ (pH 8.8). Diethylene-triaminepentaaetic acid (DTPA) was coupled to LDL by a modification of the bicyclic anhydride method of Hnatowich et al. (11). The bicyclic anhydride of DTPA (Pierce Chemical Co., Rockford, IL) was dissolved in dimethylsulfoxide (DMSO) and reacted with LDL at a molar ratio of 6.4:1. Excess DTPA was removed by thoroughly washing the LDL in microconcentrators with 0.1 N NaHCO₃.

The buffer was changed to 0.1 M NaAcetate/0.02 M NaCitrate, pH 5, and $^{111}$InCl₃ (Medi-Physics, Inc., Emeryville, CA) was added with mixing. After washing the $^{111}$In-LDL with NaAcetate/NaCitrate buffer, the buffer was changed to 0.05 M NaAcetate/0.15 M NaCl, pH 7.4. Samples were filtered (Millex GV 0.22-μm filter units, Millipore Corp., Bedford, MA) to remove precipitated LDL and to sterilize.

LDL was labeled with $^{125}$I by a modification of the McFarlane method (14,16). Samples were dialyzed exhaustively against normal saline, pH 7.4, containing 0.1 mg/ml EDTA. Samples were filter sterilized with Millex GV filter units (0.22 μm).

A determination of the average number of DTPA groups bound per LDL in the bicyclic anhydride of DTPA procedure was performed using a modification of the method of Hnatowich et al. (11). Aliquots were taken following the reaction of the bicyclic anhydride of DTPA with LDL, and labeling with $^{111}$In was performed without removing excess DTPA. This was accomplished by adding excess 0.1 M NaAcetate/0.02 M NaCitrate, pH 5, buffer followed by addition of $^{111}$InCl₃ with mixing. The preparation was transferred to a microconcentrator precontacted with albumin and washed thoroughly with buffer. The percentage of $^{111}$In activity bound to LDL was determined. The average number of DTPA groups per LDL could then be calculated from this percentage and the known molar ratio of the bicyclic anhydride of DTPA and LDL.

Agarose Gel Electrophoresis

Electrophoresis was performed on 1% agarose gel films (Corning Medical Diagnostics, Medfield, MA) in 0.05 M NaBarbital buffer, pH 8.6, for 15 min. Gels were dried in a 55°C oven for 15–20 min, and then cut into 10 strips for counting of radioactivity. Our determination of the width and location of the strips to be cut and counted relative to the origin was based on our previous experience with $^{111}$In-labeled and unlabeled LDL, using Fat Red 7B staining. The origin was within strip 3, which extended from 8 mm to the left of the origin to 2 mm to the right of the origin. All strips were 1.0 cm wide, except for 1 cm (2.0 cm), 5 (0.5 cm), and 10 (1.5 cm). Radioactivity was counted in a NaI well counter, and the percentage of total activity in each strip was determined.

Analysis of Samples by Ultracentrifugation

Aliquots of $^{111}$In-LDL were added to unlabeled LDL and the density of the solution adjusted to 1.063 prior to centrifugation in a Beckman L2-65B ultracentrifuge for 20 hr at 10°C. The top 2-ml layer was removed and counted, as was the remainder of the tube. Control preparations of $^{111}$In-DTPA were similarly analyzed. The percentage of total activity recovered in this top layer in each case was determined.

Fibroblast Binding Studies

Human skin fibroblasts (frozen at -70°C after three passages) were thawed and grown to sub-confluence in 10% fetal bovine serum in Dulbecco's modified Eagle's medium. The medium was changed to 10% human lipoprotein-deficient serum at 48 hr prior to the study. Determination of the binding of $^{111}$In-LDL to the fibroblasts, and of $^{125}$I-LDL to the fibroblasts in the presence and in the absence of unlabeled LDL or $^{111}$In-LDL, was performed by a modification of the method of Goldstein et al. (17). Following an incubation of 4 hr at 4°C, cells were washed three times with a solution containing 50 mM Tris-chloride (pH 7.4), 0.15 M NaCl, and 2 mg/ml bovine albumin. Cells were then washed three times with a solution containing 50 mM Tris-chloride (pH 7.4) and 0.15 M NaCl. Determination of cell protein in each well was performed after digestion of the cells with 0.1 N NaOH (15). Samples were counted following the experiment to determine $^{111}$In activity, and were counted again, 1 mo later, to determine $^{125}$I activity following decay of the $^{111}$In activity.

In Vivo Studies

Male New Zealand white rabbits, 3.0–4.0 kg, were fed either a normal rabbit diet or a diet with 1.5% cholesterol added (Bio-Serv, Inc., Frenchtown, NJ) for 8 wk. Plasma cholesterol levels of normal (n = 3) and hypercholesterolemic (n = 4) animals were determined during that period and just prior to injection with radioiodinated LDL. Animals were anesthetized with intramuscular injections of Rompun (5 mg/kg) and ketamine (35 mg/kg). A marginal ear vein was used for injections of $^{111}$In-LDL. Animals were injected with 176 μg (72 μCi) of $^{111}$In-LDL and 27 μg (6 μCi) of $^{125}$I-LDL per animal, except for the images displayed in Figure 3. These images were obtained in a separate experiment in which rabbits were injected with higher levels of activity: 165 μCi (normal rabbit) and 521 μCi (hypercholesterolemic rabbit) of $^{111}$In-LDL. In addition, the hypercholesterolemic animal shown in Figure 3 was fed a cholesterol-enriched diet for a shorter interval than those above and was not atherosclerotic.

Blood samples were taken from the central ear artery at timed intervals following injection. Samples were counted in a NaI well counter and plotted as the fraction of radioactivity/ml in the 5-min sample. Data were fitted to the sum of two exponentials, and the fractional catabolic rates (FCR) were calculated by the method of Mathews (18).

Scintigraphic imaging was performed at 15 min, 2 hr, 6 hr, 24 hr, 48 hr, and 144 hr following injection. Animals were anesthetized with Rompun (5 mg/kg) and ketamine (35 mg/kg) prior to imaging. Imaging was performed in the anterior projection using a gamma camera equipped with a medium-
energy collimator and with data acquisition on a Medical Data Systems A2 computer (Medical Data Systems, Ann Arbor, MI). Images were acquired for 5 min at the early time points and 10–20 min at the later time points. Liver/heart activity ratios were determined by selection of regions of interest (ROIs) (6 x 6 pixels) over liver, heart, and background regions. Data at 144 hr after injection were excluded because background activity was ≥50% of heart activity in the majority of animals owing to significant blood-pool clearance at this time point. Animals were killed by i.v. injection of pentobarbital (100 mg/kg). Tissue samples from various organs were removed, blotted dry, weighed, and counted. Standards were also counted, and the % of injected dose/gm of tissue was determined for each organ.

RESULTS

In Vitro Studies

DTPA was bound to LDL isolated from human hyperlipidemic plasma by reacting the bicyclic anhydride of DTPA with LDL at a molar ratio of 6.4:1. At this starting molar ratio of reactants, the average number of DTPA groups per LDL was found to be 1.2. Following labeling of the LDL-DTPA preparations with $^{111}$In, the radiolabeled preparations were analyzed by agarose gel electrophoresis. Control samples of $^{111}$In-colloid and $^{111}$In-DTPA were analyzed together with $^{111}$In-LDL by agarose electrophoresis. The gels were cut into strips and the radioactivity counted. Figure 1 compares the migration of $^{111}$In-LDL with the control samples. Indium-$^{111}$In-LDL (Fig. 1A) migrated as a single band and did not demonstrate any significant contamination with $^{111}$In-DTPA (Fig. 1B) or $^{111}$In-colloid (Fig. 1C).

Aliquots of $^{111}$In-LDL samples were added to unlabeled LDL and analyzed by ultracentrifugation at density = 1.063. The top 2-ml of a 6-ml volume was removed and counted. Ninety-one percent of the radioactivity was found in this fraction. In contrast, when a control sample of $^{111}$In-DTPA was analyzed, only 27% of the radioactivity was found in this top layer. After refrigeration and storage of the $^{111}$In-LDL preparation for 9 days, a repeat analysis demonstrated 84% of the radioactivity in the LDL fraction.

The $^{111}$In-LDL preparation was assayed for retention of native biologic activity using cultured human fibroblasts. The ability of $^{111}$In-LDL to competitively inhibit binding of $^{125}$I-LDL to LDL receptors was compared with that of unlabeled LDL. As shown in Figure 2A, $^{111}$In-LDL demonstrated significant inhibition of $^{125}$I-LDL binding to receptors (80% inhibition with 100 μg of $^{111}$In-LDL), although the inhibition of binding was not as pronounced as with unlabeled LDL (88% inhibition with 100 μg of unlabeled LDL). The results of direct binding of $^{111}$In-LDL and $^{125}$I-LDL to fibroblasts are shown in Figure 2B. Although the two radiotracers were not studied at the same concentrations, $^{125}$I-LDL binding appeared to reach a plateau at 25 μg while $^{111}$In

FIGURE 1

Agarose gel electrophoresis of $^{111}$In-LDL (A), $^{111}$In-DTPA (B), and $^{111}$In-colloid (C). Gels were cut into 10 strips. Data are expressed as % of total activity on gel.

LDL binding demonstrated lower affinity and did not appear to reach a plateau even at 100 μg. Thus, $^{111}$In-LDL demonstrated an increased nonsaturable component. These results suggest that while $^{111}$In-LDL is recognized by the LDL receptors, it behaves as a modified LDL that cannot interact fully with these receptors.

In Vivo Studies

Male New Zealand white rabbits were fed either a normal rabbit diet (n = 3) or a diet with 1.5% choles-
terol added (n = 4) for 8 wk. The mean plasma-
cholesterol level for the animals fed a cholesterol-sup-
plemented diet was 1,539 mg/100 ml (vs. 25 mg/100
ml for normal rabbits). Blood samples were drawn from
normal (n = 3) and hypercholesterolemic rabbits (n =
4) at frequent intervals following the i.v. injection of
125I-LDL and 111In-LDL. The samples were weighed
and counted to determine the radioactivity per milliliter
of blood. The fractional catabolic rate (FCR) were
calculated using a two-compartment model. The FCR
values for 125I-LDL were 2.08 ± 0.16 pools/day in
normal rabbits and 0.74 ± 0.20 pools/day for hyper-
cholesterolemic rabbits. For 111In-LDL, the FCR values
were 1.52 ± 0.20 pools/day in normal rabbits and 1.12
± 0.26 pools/day for hypercholesterolemic rabbits. The
FCR of 125I-LDL was significantly lower in hypercho-
lesterolemic rabbits than in normal rabbits (p < 0.001).
The FCR of 111In-LDL was lower in hypercholesterol-
emic rabbits than in normal rabbits, although the
difference was of borderline significance (p < 0.08). An
indication of the rate of blood-pool clearance and he-
ptic uptake of 111In-LDL was also obtained noninva-
sively by scintigraphic imaging (Fig. 3). Animals were
imaged at intervals from 15 min to 6 days after injec-
tion. Regions of interest in the computer-acquired im-
gages were placed over the heart, as a measure of blood-
pool activity, and over the liver. The liver/heart ratio
of activity per pixel was determined. The liver/heart
ratios (Fig. 4) demonstrate a faster rate of blood-pool
 clearance in normal rabbits than in hypercholesterol-
emic rabbits.

Animals were sacrificed at 6 days after injection with
radiolabeled LDL. Biodistribution data (expressed as %
of injected dose/g of tissue) are listed in Table 1. The
overall levels of uptake in all tissues, obtained from
both normal and hypercholesterolemic rabbits, were
many times higher with 111In-LDL than with 125I-LDL.

For example, in normal rabbits 111In-LDL uptake in
adrenal gland was 35 times higher than 125I-LDL up-
take, and uptake in liver was 15 times higher. Indium-
111-LDL uptake in liver and adrenal glands was de-
creased in hypercholesterolemic rabbits, compatible
with downregulation of LDL receptors in these chole-
terol-fed animals. As shown in Figure 5, the response
of the adrenal glands to hypercholesterolemia was quite
marked with 111In-LDL, with hypercholesterolemic rab-
bits demonstrating only 14% of the uptake found with
normal rabbits (p < 0.001). Uptake by the liver in
hypercholesterolemic rabbits was 57% of that present
in normal rabbits (p < 0.02). In contrast, hypercholes-
terolemic rabbits demonstrated a greater than two-fold
increase in uptake of 111In-LDL in the proximal aorta,
the site of the most extensive atherosclerotic plaques,
in comparison with normal rabbits (p < 0.02).

**DISCUSSION**

The development of radiolabeled LDL for the non-
invasive detection of the extent of early atherosclerotic
disease would allow the noninvasive evaluation of treat-
ments aimed at reducing lipoprotein uptake by blood
vessels. However, there are serious drawbacks associ-
ated with each of the currently reported methods (5–
7). The low-energy photon of 123I is a poor label for
external imaging. The half-lives of 99mTc and 123I are
too short for effective imaging of uptake into athero-
sclerotic plaques, owing to the slow plasma clearance
of LDL (9). Consequently, we have directed our efforts
to developing 111In-labeled LDL, in order to take ad-
vantage of the more suitable physical characteristics
of this isotope.

When binding DTPA to LDL or to any protein, and
radiolabeling with 111In, the possibility of contamina-
tion with 111In-colloid or with 111In-DTPA must always
be considered. Analysis by agarose gel electrophoresis
demonstrated no significant contamination in our preparation. Furthermore, ultracentrifugation of the $^{111}$In-LDL preparation demonstrated that nearly all of the radioactivity was bound to a product with the density of native LDL.

The $^{111}$In-LDL preparation was assayed for retention of biologic activity in a fibroblast cell culture system. The radiolabeled material was found to be able to competitively inhibit the uptake of $^{125}$I-LDL by the LDL receptors expressed on fibroblasts. However, the inhibition was not as pronounced as with unlabeled LDL at equal concentrations. In addition, the uptake of $^{111}$In-LDL by the fibroblasts did not appear to be fully saturable. These results suggest that while $^{111}$In-LDL is recognized by LDL receptors, it is clearly a modified LDL and nonreceptor mediated mechanisms are also operative. The $^{111}$In-LDL preparations used in these experiments contained an average of 1.2 DTPA groups per LDL. DTPA binds to LDL via lysine amino groups, and has the potential to interfere with receptor-mediated binding of LDL (19). That portion of the LDL preparation with an above-average number of DTPA groups per molecule may not have been recognized as strongly by the LDL receptors, and may have contributed to the reduced affinity observed for the whole preparation for these receptors. Future experiments will be performed with LDL that has been reacted with lower concentrations of the cyclic anhydride of DTPA, so as to result in a lower average number of DTPA groups per molecule. This may result in a preparation with a greater affinity for the specific receptor. Studies with $^{111}$In-labeled anti-tumor antibodies labeled by a similar method have shown that loss of biologic activity is proportional to the number of DTPA groups bound.
per molecule (20). However, for the purpose of imaging atherosclerotic lesions, retention of specificity and high affinity for LDL receptors may not be important. In fact, it has been reported that LDL enters the vascular subintimal space by nonspecific pathways (21). Consequently, we will study the imaging characteristics of LDL preparations with an increased number of DTPA groups per LDL as well as preparations with a decreased number of DTPA groups.

The FCR values calculated from the disappearance of $^{11}$In-LDL from the blood-pool compartment revealed a lower value for hypercholesterolemic rabbits than for normal rabbits. This was the expected relationship because of the downregulation of LDL receptors in response to hypercholesterolemia (22,23). This difference was not as great as that demonstrated for the two groups of rabbits using $^{125}$I-LDL, however, and was of borderline significance ($p < 0.08$). The results are concordant with the fibroblast binding studies, which indicated some loss of LDL receptor interaction by $^{11}$In-LDL. In contrast, the biodistribution data indicated that $^{11}$In-LDL behaved very much like an unmodified LDL. Uptake in the adrenal gland of hypercholesterolemic rabbits was only 14% of that seen with normal rabbits, while hepatic uptake of LDL was reduced by 43% in the cholesterol-fed rabbits. Since the adrenal gland has the highest concentration of LDL receptors per gram of tissue (24), these results reflect downregulation of these receptors, and suggest that binding to specific receptors is an important component of $^{11}$In-LDL uptake. Finally, whether by receptor or nonreceptor pathways, increased uptake of $^{11}$In-LDL in the atherosclerotic proximal aorta was demonstrated in the cholesterol-fed rabbits.

Our biodistribution studies with $^{11}$In-LDL demonstrate significantly higher levels of uptake in all tissues in comparison with $^{125}$I-LDL. Similar results were reported by Vallabhajosula et al. (8) in their comparison of $^{99}$Tc-LDL uptake with that of $^{125}$I-LDL and $^{125}$I-tyramine cellulose-LDL. Radioiodinated tyramine cellulose-LDL has been demonstrated to act as an intracellularly trapped ligand (25), a radiotracer that is not significantly released over the time course of the experiment and whose biodistribution reflects total uptake. Vallabhajosula et al. (8) suggested that $^{99}$Tc-LDL also acts as an intracellularly trapped ligand. The levels of

<table>
<thead>
<tr>
<th>Organ</th>
<th>$^{11}$In-LDL*</th>
<th>$^{125}$I-LDL*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hyper</td>
</tr>
<tr>
<td>Blood</td>
<td>0.008 ± 0.003</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td>P-Aorta</td>
<td>0.010 ± 0.003</td>
<td>0.025 ± 0.007</td>
</tr>
<tr>
<td>M-Aorta</td>
<td>0.011 ± 0.004</td>
<td>0.016 ± 0.003</td>
</tr>
<tr>
<td>D-Aorta</td>
<td>0.009 ± 0.001</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td>Lung</td>
<td>0.017 ± 0.008</td>
<td>0.039 ± 0.020</td>
</tr>
<tr>
<td>Liver</td>
<td>0.200 ± 0.021</td>
<td>0.113 ± 0.037</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.598 ± 0.075</td>
<td>0.481 ± 0.130</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.149 ± 0.022</td>
<td>0.167 ± 0.047</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.594 ± 0.067</td>
<td>0.085 ± 0.018</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.005 ± 0.002</td>
<td>0.004 ± 0.004</td>
</tr>
<tr>
<td>Bone-C</td>
<td>0.006 ± 0.002</td>
<td>0.009 ± 0.002</td>
</tr>
<tr>
<td>Bone-M</td>
<td>0.129 ± 0.034</td>
<td>0.389 ± 0.112</td>
</tr>
<tr>
<td>Intest.-S</td>
<td>0.017 ± 0.003</td>
<td>0.014 ± 0.009</td>
</tr>
<tr>
<td>Intest.-L</td>
<td>0.010 ± 0.003</td>
<td>0.010 ± 0.005</td>
</tr>
</tbody>
</table>

* Data expressed as mean ± s.d. of % of injected dose/g tissue.

Hypercholesterolemic rabbits, n = 4; Normal rabbits, n = 3.

Abbreviations: P-Aorta = proximal aorta; M-Aorta = mid-aorta; D-Aorta = distal aorta; Bone-C = bone cortex; Bone-M = bone marrow; Intest.-S = small intestine; and Intest.-L = large intestine.

![TABLE 1](image)

**FIGURE 5**

Uptake of $^{11}$In-LDL (% of dose/g) in hypercholesterolemic rabbit tissues displayed as % of uptake (% of dose/g) observed in the same tissues in normal rabbits. PA = proximal aorta; LI = liver; SP = spleen; KI = kidney; and AD = adrenal.
tissue accumulation of $^{111}$In-LDL that we observed were slightly lower than the levels those authors reported for $^{99m}$Tc-LDL, but were still many times higher than those with $^{125}$I-LDL. Our results with $^{111}$In-LDL are closer to those reported for $^{99m}$Tc-LDL by Lees et al. (7). Their studies with $^{99m}$Tc-LDL and our studies with $^{111}$In-LDL utilized human LDL, while Valla and others utilized rabbit LDL in their studies. This may partially account for our slightly lower levels of uptake with $^{111}$In-LDL. In addition, our biodistribution data were obtained 6 days after injection, while the other studies were performed at only 16 and 24 hr after injection. A slow leakage from cells of radiiodinated tyramine cellulose-LDL has been reported by Pittman et al. (25), and a similar process may have occurred with $^{111}$In-LDL. Taking into account the different behavior of human LDL and rabbit LDL, and the different time periods involved, our data suggest that, like $^{99m}$Tc-LDL, $^{111}$In-LDL behaves as an intracellularly trapped ligand.

Our data demonstrate decreased uptake of $^{111}$In-LDL in liver and adrenal of hypercholesterolemic rabbits as compared to control animals, reflecting downregulation of LDL receptors in the hypercholesterolemic animals. The same phenomenon could not be demonstrated with $^{125}$I-LDL, probably as a result of catabolism and loss of radiolabel during the six-day experiment. Retention of a high level of radiotracer uptake represents one of the advantages of labeling LDL with $^{111}$In, because relatively long experiments are necessary owing to the slow plasma clearance of LDL.

Scintigraphic imaging was performed, which demonstrated that the rate of blood-pool clearance and hepatic uptake of $^{111}$In-LDL could be evaluated non-invasively by calculation of liver/heart activity ratios. The rate of blood-pool clearance was higher in normal rabbits than in hypercholesterolemic rabbits as expected (22,23). The greatest accumulations of activity in the images were noted in liver and spleen. Atherosclerotic plaques were not visualized in these experiments in which animals were injected with only 72 µCi of $^{111}$In-LDL. However, even at this low level of activity, the rate of blood-pool clearance and hepatic uptake could be evaluated scintigraphically.

We have demonstrated that $^{111}$In-LDL is taken up into the atherosclerotic aortas of hypercholesterolemic rabbits and is retained at six days after injection, by which time blood and background levels of activity had reached low levels. Efforts to image atherosclerotic lesions in human studies with $^{99m}$Tc-LDL (10) have had only limited success because the relatively long half-life of LDL in the blood pool makes it difficult to image LDL labeled with an isotope with a short half-life. Indium-111, with a longer half-life, has a significant theoretical advantage over $^{99m}$Tc for imaging with radiolabeled-LDL. Our results in a rabbit model suggest that $^{111}$In-LDL has the potential to be a useful agent for external imaging of atherosclerotic lesions and of lipoprotein biodistribution.

REFERENCES


