Radiolabeled Cholesteryl Iopanoate/ Acetylated Low Density Lipoprotein as a Potential Probe for Visualization of Early Atherosclerotic Lesions in Rabbits

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Purpose. Atherosclerosis is the underlying factor leading to such cardiovascular diseases (CVD) as stroke, aneurysm, and myocardial infarction. The early detection of atherosclerotic plaques is considered to be crucial for successful prevention and/or therapeutic and dietary intervention of CVD. Current diagnostic practice, on the other hand, can only detect the problem at an advanced stage. The purpose of this study was to examine the potential of using a radiolabeled cholesterol ester analog/acetylated low density lipoprotein (AcLDL) conjugate as a diagnostic agent for the early and non-invasive detection of atherosclerosis and for the monitoring of the effects of drug therapy.

Methods. Cholesteryl iopanoate (CI), a cholesterylester analog, was synthesized, radiolabeled, and incorporated into AcLDL. Early atherosclerotic lesions were induced in New Zealand White rabbits. ^{125}I-CI/AcLDL was injected intravenously at 2 μCi/kg. Blood samples were taken at different time intervals after injection and clearance of the injected drug from blood was studied. The rabbits were sacrificed after 72 hours and the distribution of radioactivity in various organs was investigated. Aortae of both atherosclerotic lesion and control rabbits were removed for Sudan IV staining and autoradiography in order to confirm the formation of the atherosclerotic lesion and localization of radioactivity.

Results. The injected drug was found to be cleared from blood following a two compartment model. Radioactivity in the atherosclerotic aorta was found to be about 8 times higher than that in normal aorta, suggesting that the proposed diagnostic probe was selectively taken up by the atherosclerotic lesion. The autoradiography and staining confirmed that the localization of the proposed probe was superimposed with the atherosclerotic lesion site.

Conclusions. The results suggested that incorporation of CI into AcLDL resulted in the selective localization of CI at the atherosclerotic plaque areas. CI/AcLDL labeled with appropriate radioisotope has the potential to be used as a probe for visualization of early atherosclerotic lesion using scintigraphy technology.

KEY WORDS: AcLDL; diagnostic agent; atherosclerosis; visualization; autoradiography; cholesteryl iopanoate.

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INTRODUCTION

The pathogenesis of atherosclerosis is a long-term process developed in a complex inflammatory and hyperlipidemic environment (1). It is known that therapeutic and/or dietary intervention is usually successful at an early stage of atherosclerosis. However, angiography, which is the standard method for the detection of arterial obstruction and stenosis, can only identify advanced atherosclerotic lesions which usually require surgical intervention. It has also been suggested that the atherosclerosis is usually more severe than the current diagnostic method predicts (2,3). Therefore, it is important to develop an early and non-invasive detection of atherosclerosis to permit a successful therapeutic and dietary intervention.

Although the etiology of atherosclerosis is not fully understood, the involvement of monocyte-macrophages in the arterial intima and their subsequent accumulation of modified low density lipoprotein (LDL) have been well elucidated (4). Macrophages in the subendothelial space accumulate cholesteryl esters to become foam cells (1,4), and foam cells with massive accumulation of cholesteryl esters were observed in atheromatous plaques (5). It is known that macrophages express a scavenger receptor for modified LDL such as oxidized LDL and acetylated LDL (AcLDL) (6), and that such a receptor was found to be involved in the formation of macrophage foam cell *in vivo* (7). The potential for using LDL with its apo B-100 protein radiolabeled, including ^{123}I-LDL (17,18), ^{125}I-LDL (19), ^{111}In-LDL (20,21), and ^{99m}Tc-LDL (22–24), as radiomarkers for the detection of atherosclerosis has been investigated. However, when LDL is taken up by the targeted cells, it is quickly degraded by lysosomal enzymes and excreted.

To overcome this problem, we have chosen an iodinated cholesteryl ester analogue, cholesteryl iopanoate (I-CI), as a radiotracer. It has been demonstrated that I-CI is resistant to hydrolysis *in vivo* (8,14) and thus is able to accummulate in the targeted tissues. In addition, since each LDL particle contains 1,500 cholesteryl ester molecules in its oily core, incorporation of hydrophilic radiotracers can provide more radioactivity than labeling LDL on its apo B-100 protein. Based on the role of macrophages in the formation of atherosclerosis, we examined the use of AcLDL as a lesion-seeking atherogenic carrier to deliver CI to the site of the early lesions for the purpose of early diagnosis of atherosclerosis. New Zealand White rabbits were used and early atherosclerosis was successfully induced. The localization of the proposed diagnostic probe at the early lesion site was examined and compared with healthy rabbits.

MATERIALS AND METHODS

Materials

Thin layer chromatography (TLC) was carried out on silica gel-60, F-254 polyethylene-backed plates (Fisher Scientific) and visualized by UV. Column chromatography was performed on silica gel-60 (230–400 mesh) (Aldrich). High Performance Liquid Chromatography (HPLC) analyses were performed using a Beckman system with a Bondclone 10 C18, 150 × 3.0 mm column (Phenomenex). Radioactivity was measured using a CKB-WALLAC 1277 GAMMAMASTER automatic gamma

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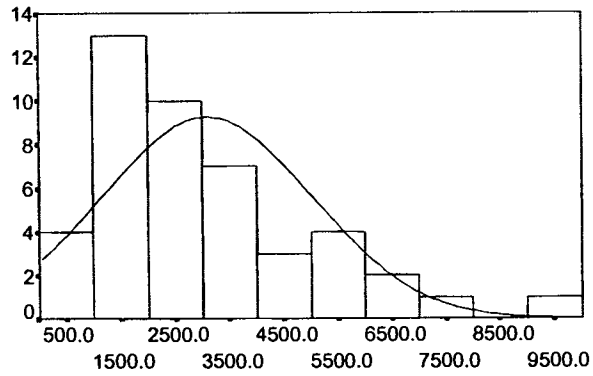
counter. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a Mini-PROTEIN® II Electrophoresis Cell (Bio-Rad). Image analysis was done by BIOQUANT™ System IV.

Carrier free aqueous Na^{125}I was purchased from Mandel Scientific Company Ltd. Pivalic acid was obtained from Aldrich Chemical Co. Canada. Sephadex® G-25 was purchased from Pharmacia Biotech AB, Uppsala, Sweden. Poly-Prep® chromatography columns were obtained from Bio-Rad Laboratories (Hercules, CA). SDS-PAGE molecular weight standards and Bio-Rad protein assay standards were purchased from Bio-Rad Laboratories (Richmond, CA). L-α-phosphatidylcholine dipalmitoyl (1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine, DPPC) and DL-α-phosphatidylethanolamine dipalmitoyl (1,2-dihexadecanoyl-*rac*-glycero-3-phosphoethanolamine, DPPE) were purchased from Sigma Chemical Co. Canada. Seal oil was obtained from Terra Nova Fishery, Newfoundland, Canada.

Methods

Preparation of AcLDL

Fresh human plasma was obtained from the Canadian Red Cross. LDL was isolated as previously described (9) by the sequential ultracentrifugation of fresh human plasma at 40,000 rpm for 24–40 hours at 8°C using a Beckman L8-M ultracentrifuge and a 60 Ti rotor. All LDL preparations were dialysed at 4°C overnight against a buffer containing 0.3 mM EDTA, 150 mM NaCl and 50 mM Tris (pH 7.4). Acetylation of LDL was performed according to the method of Basu and Goldstein (11). The AcLDL preparation thus obtained was dialysed at 4°C as described above. Protein concentrations were determined by the Bradford protein assay method with BSA as the standard (10).



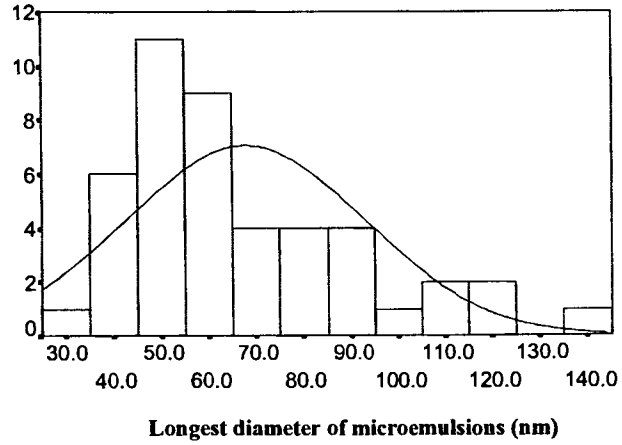


Fig. 1. Image analysis of seal oil microemulsion.

Preparation of ^{125}I-CI

CI was synthesized as described (12) and radiolabeled with ^{125}I via an iodine exchange reaction in a pivalic acid melt (13). The chemical and radiochemical purity of ^{125}I-CI were determined by HPLC and TLC (14).

Incorporation of ^{125}I-CI into AcLDL

A mixture of 12 mg DPPC, 8 mg DPPE, 20 mg seal oil and 1 mg ^{125}I-CI in chloroform was dried with a gentle stream of nitrogen, and re-suspended in 10 mL of saline. The suspension was sonicated for 2 hours using a Virosonic Cell disrupter Model 16-850 at 40–50 watts at about −10°C. The mixture was then centrifuged at 40,000 rpm for 20 hours at 4°C in a Beckman SW41 rotor. The microemulsion that floated to the top of the centrifuge tube was collected and subjected to image analysis by a BIOQUANT™ System IV (Fig. 2). Microemulsion thus obtained was incubated with AcLDL at 37°C for 24 hours. The resultant mixture was centrifuged again to obtain ^{125}I-CI/AcLDL.

Characterization of ^{125}-I-CI/AcLDL

Column chromatography. After separation from microemulsion by ultracentrifugation, ^{125}I-CI/AcLDL (about 2 mg/mL), 500 μL, was loaded onto a Poly-Prep® chromatography column packed with about 1 g of Sephadex® G-25 and eluted with phosphate buffered saline (PBS pH = 7.4). Fractions of 0.5

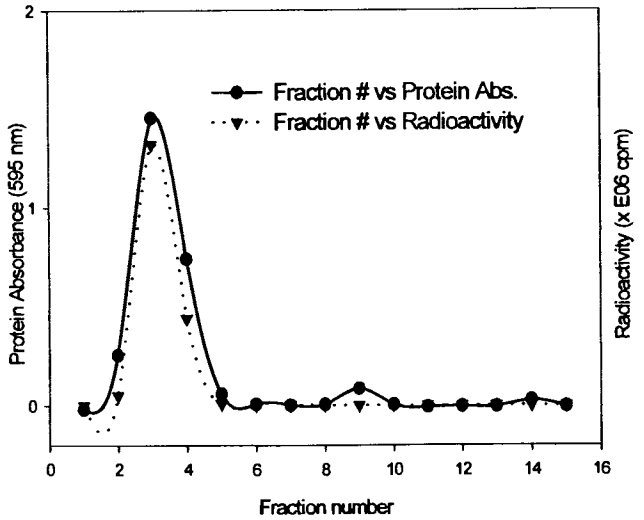


Fig. 2. Chromatograms of gel filtration of ^{125}I-CI/AcLDL preparation with respect to its radioactivity and protein concentration.

mL each were collected. The radioactivity and protein content associated with each fraction were determined and plotted.

Electrophoresis. In order to assess the integrity of the apo B-100 protein associated with ^{125}I-CI/AcLDL, the ^{125}I-CI/AcLDL sample was examined by SDS-PAGE and compared with native LDL and AcLDL as reported (15).

Stability of ^{125}I-CI/AcLDL in Plasma. ^{125}I-CI/AcLDL was incubated with plasma according to a reported method (16) with modifications. ^{125}I-CI/AcLDL, 1 mL, was incubated with 5 mL of human plasma at 37°C for 24 hours. Different fractions including very low density lipoprotein (VLDL), LDL, high density lipoprotein (HDL), and lipoprotein deficient serum (LPDS) were separated as previously described (9). The radioactivity in each fraction was determined.

Animal Model and Protocol

Eight female New Zealand White rabbits (3.20–4.75 kg) were randomly divided into two groups. One group was maintained on normal rabbit chow (n = 4) and the other group on rabbit chow supplemented with 1% of cholesterol and 2% of lard (n = 4) for six weeks to induce the development of early stage atherosclerotic lesions. The ^{125}I-CI/AcLDL preparation was then injected via the marginal ear vein to both control and atherosclerotic lesion rabbits at 2 μCi/kg. Blood samples were taken at 5 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours and 72 hours after injection. The rabbits were then sacrificed and 4% formaldehyde buffer was infused into the heart to fix the organs. Tissues, including aorta, liver, spleen, lung, kidney and heart, were removed for determination of radioactivity using a gamma counter.

Aorta Staining and Autoradiography

The aortae which were free of fat and connective tissues were opened longitudinally, and fixed in 4% formaldehyde for 48 hours. After fixation, the aortae were stained with Sudan IV solution, and subsequently exposed to Kodak X-OMAT™ AR film and stored in Kodak X-Omatic cassette at −70° for one week.

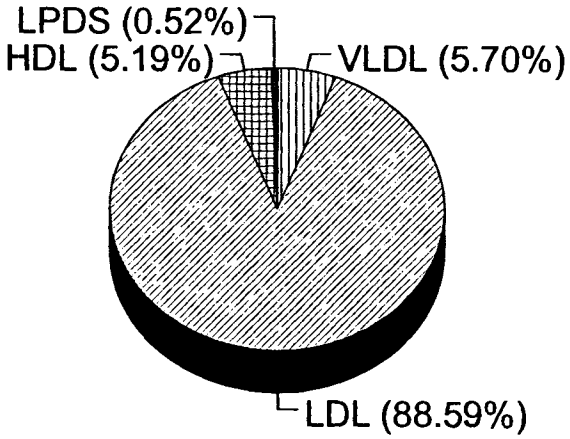


Fig. 3. Distribution of radioactivity upon incubation of ^{125}I-CI/AcLDL with plasma. The values are % of initial radioactivity associated with individual fractions after incubation.

Table I. Comparison of Cholesterol, Triglyceride, HDL, and LDL Contents in Blood Between Atherosclerotic and Control Rabbits

	Atherosclerotic group	Control group
Cholesterol (mmol/L)	60.01 ± 8.79	0.57 ± 0.12
Triglyceride (mmol/L)	4.90 ± 2.67	0.69 ± 0.04
HDL (mmol/L)	18.65 ± 3.00	0.37 ± 0.01
LDL (mmol/L)	39.13 ± 4.57	0.16 ± 0.08

Note: Average ± SEM.

RESULTS AND DISCUSSION

It is clear that macrophages play an important role in the formation of atherosclerotic lesions (via the formation of the foam cells) (1,4,5,7) and they express scavenger receptors which mediate the uptake of AcLDL. It is believed that CI incorporated into AcLDL (CI/AcLDL) is taken up by macrophages (25) through the scavenger receptor pathway. We have, therefore, examined the potential of using AcLDL to deliver CI to early atherosclerotic lesions induced in rabbits. CI is easily labeled with ^{131}I or ^{123}I, and will allow the visualization of the lesion sites using gamma scintigraphy technology if the proposed method can selectively target CI to the lesion sites.

Preparation of ^{125}I-CI/AcLDL

^{125}I-CI was synthesized and its radiochemical purity was found to be more than 96%. Microemulsions have been widely employed in loading drugs into LDL, and in our studies, seal oil was novelly used to form microemulsion for its high efficiency of transferring the drugs to LDL (unpublished data), presumably because seal oil contains high levels of highly unsaturated triglycerides, which may allow for high solvation of hydrophobic drugs and the increase of fluidity. Image analysis of seal oil microemulsion (Fig. 1) shows that its longest diameter

Table II. Comparison of Distribution of Radioactivity in Various Tissues Between Atherosclerotic and Control Rabbits After I.V. Injection of ^{125}I-CI/AcLDL at 2 μCi/kg

Tissue	Atherosclerotic group (n = 4) % administered dose/g tissue	Control group (n = 4) % administered dose/g tissue
Liver	0.46 ± 0.29	0.68 ± 0.068
Spleen	0.98 ± 0.87	1.04 ± 0.57
Lung	0.21 ± 0.14	0.20 ± 0.17
Heart	0.060 ± 0.017	0.062 ± 0.032
Aorta	0.056 ± 0.034*	0.0069 ± 0.0019
Kidney	0.057 ± 0.022	0.032 ± 0.017
Blood	0.087 ± 0.030*	0.013 ± 0.0073

Note: Average ± SEM.
* $p < 0.05$.

Table III. Pharmacokinetic Parameters of the Radioactivity in the Blood of Atherosclerotic and Control Rabbits After I.V. Injection of ^{125}I-CI/AcLDL at 2 μCi/kg Dose

	$k_\alpha(hr^{-1})$	$t_{1/2\alpha}(hr)$	$k_\beta(hr^{-1})$	$t_{1/2\beta}(hr)$	$V(mL.kg^{-1})$	$CL(mL.kg^{-1}.hr^{-1})$
Atherosclerotic group	2.59 ± 0.50	0.29 ± 0.054	0.012 ± 0.0014	56.60 ± 6.99	49.20	128.04
Control group	0.70 ± 0.23	1.09 ± 0.44	0.16 ± 0.0028	43.79 ± 8.59	144.23	102.80

Note: k_α,k_β, elimination constant; $t_{1/2}$, drug half life in blood; V, Volume of distribution; CL, Clearance. Average ± SEM.

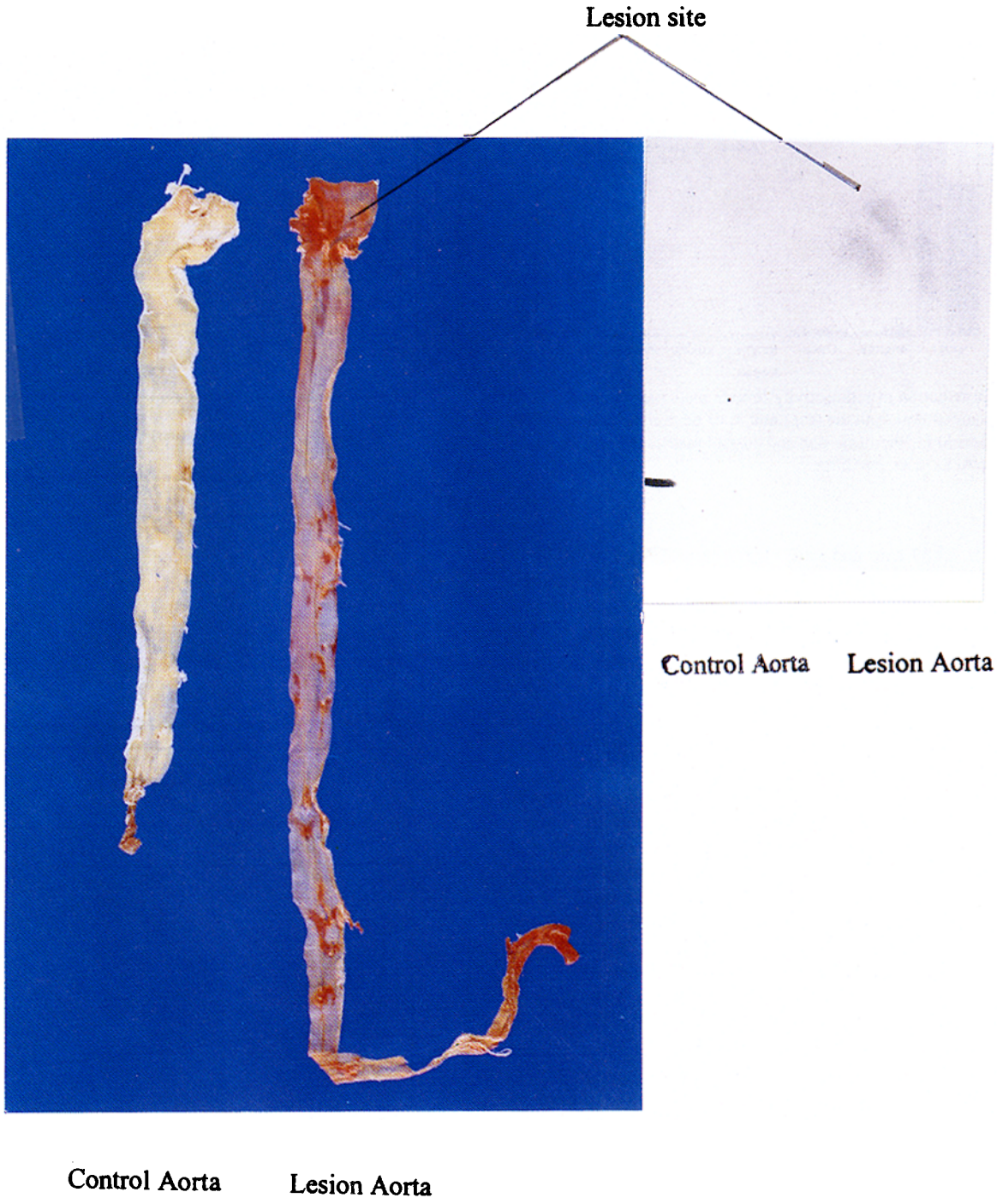


Fig. 4. Correlation of Sudan IV staining (left) and autoradiogram (right).

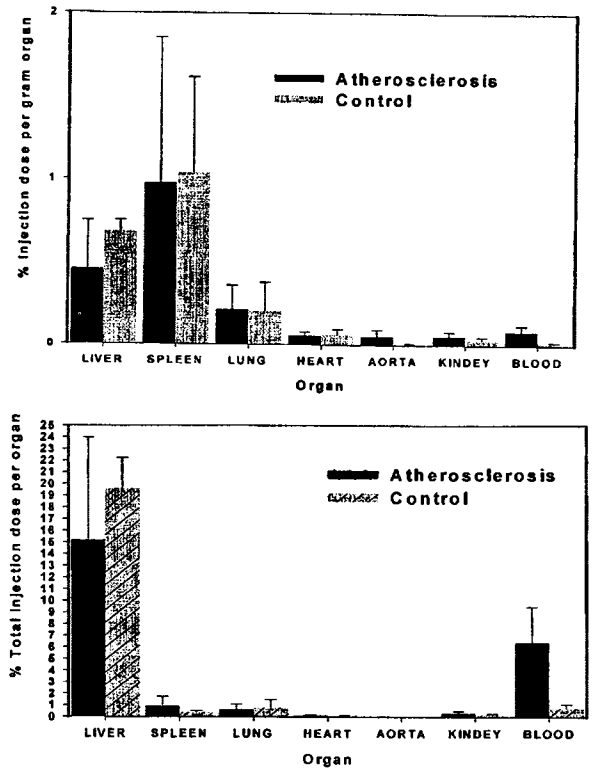


Fig. 5. Distribution of radioactivity in various tissues expressed as % of administered dose/g tissue (top) and % of administered dose/whole tissue (bottom) in atherosclerotic and control rabbits after i.v. injection of ^{125}I-CI/AcLDL at 2 μCi/kg.

= 67.8 ± 25.37 nm, and its image area = 3091.2 ± 1934.77 nm^2. With the loading method we employed, up to five hundred ^{125}I-CI molecules can be incorporated into each AcLDL particle (unpublished data). The specific radioactivity of the ^{125}I-CI/AcLDL was about 2.5 μCi/mL.

Characterization of ^{125}I-CI/AcLDL

The ^{125}I-CI/AcLDL was characterized in the following manner. After gel filtration using Sephadex® G-25 (Fig. 2), each fraction was examined with respect to radioactivity and protein content. As shown in Fig. 2, chromatograms of radioactivity and protein content were superimposed, indicating that ^{125}I-CI was either incorporated into or tightly associated with AcLDL. After incubation of ^{125}I-CI/AcLDL with human plasma at 37°C for 24 hours, the mixture was separated into different plasma fractions. The radioactivity in VLDL, LDL, HDL, and LPDS was determined. As shown in Fig. 3, about 90% of the initial radioactivity was still associated with the LDL fraction (d = 1.019–1.063 g/mL), suggesting there is only little redistribution of ^{125}I-CI from ^{125}I-CI/AcLDL to other plasma components. Human plasma cholesteryl ester transfer protein (CETP), which mediates the transfer and exchange of cholesteryl ester and triacylglycerides among different lipoprotein subspecies, has no effect on the redistribution of CI from AcLDL to other lipoproteins. This result demonstrated that the targeting potential of CI/AcLDL to macrophage scavenger receptor was not compromised by the effect of CETP. The integrity of apo B-100 in ^{125}I-CI/AcLDL is crucial for the recognition of the

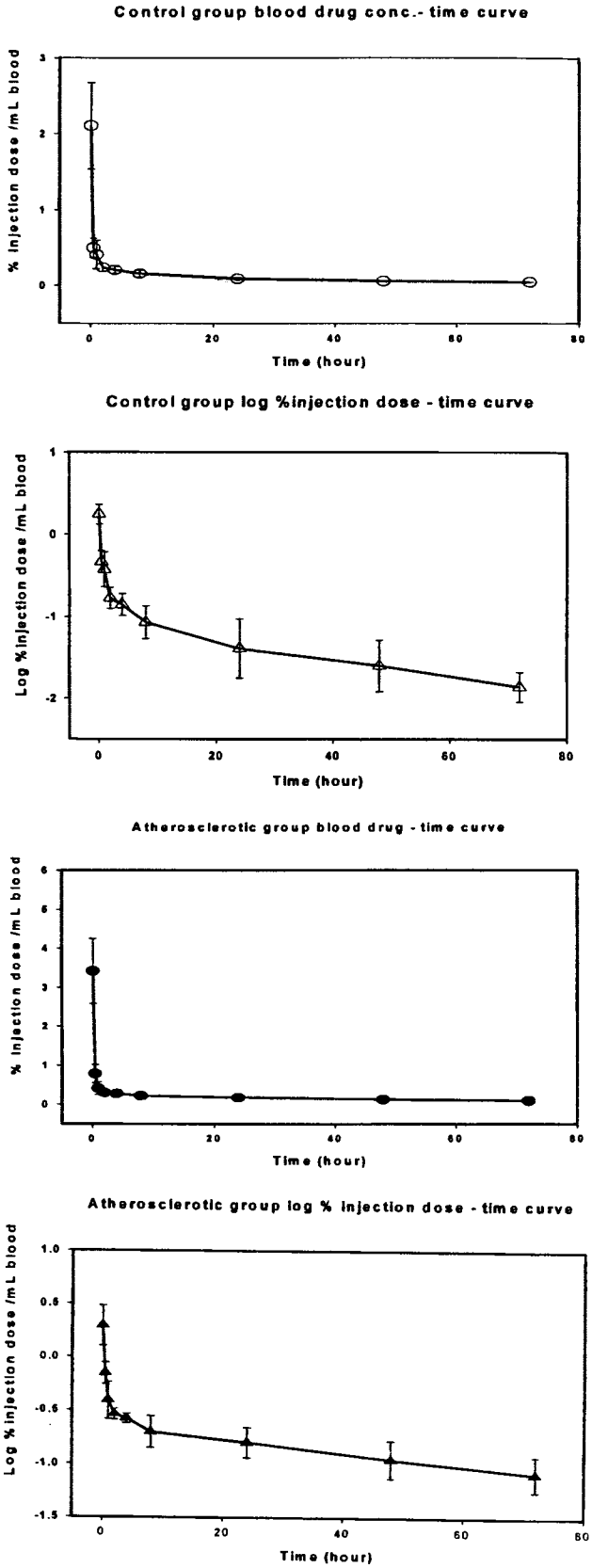


Fig. 6. Amount of ^{125}I-CI in blood versus time after i.v. injection of ^{125}I-CI at 2 μCi/kg in atherosclerotic (●,▲), and control rabbits (○,△).

particle by the scavenger receptor. To examine the integrity of the apo B-100 in ^{125}I-CI/AcLDL, the sample was subjected to 5% SDS-PAGE analysis. The ^{125}I-CI/AcLDL preparation demonstrated an identical apo B-100 band as that of native LDL and AcLDL and no fragmented protein bands were found (data not shown).

Analysis of Animal Data

Although there are many published methods on generating atherosclerotic lesions in rabbits (19,24,27,28), early atherosclerosis was induced in New Zealand White rabbits in this case by feeding a regular diet supplemented with 1% of cholesterol and 2% of lard for six weeks while the control group was maintained on a regular diet. Cholesterol, triglyceride, HDL, and LDL levels in blood were measured in both control and atherosclerotic groups. The results are summarized in Table I. The atherosclerotic group demonstrated increased levels of all lipids measured. ^{125}I-CI/AcLDL was then given intravenously to both control and atherosclerotic groups at 2 μCi/kg. The radioactivity in blood samples taken at different time intervals was measured. The results presented in Fig. 6 and Table III demonstrate that the clearance of ^{125}I-CI/AcLDL from blood follows a two compartment model with $t_{1/2\alpha}$ of 0.28 hr and 1.09 hr and $t_{1/2\beta}$ of 56.60 hr and 43.79 hr for atherosclerotic and control groups, respectively. The difference in $t_{1/2}$ values between the two groups is not significant ($p > 0.05$).

The tissue distribution studies suggested that there were no significant difference between the atherosclerotic group and control group in all tissues with exception of the aorta and blood (at 72 hours). The radioactivity in both aorta and blood expressed as percent of injected dose per gram of organ, was about 8 times higher for the lesion group than for the control group (Fig. 5 and Table II). The increased radioactivity level in the aorta of atherosclerotic rabbits was likely due to the higher level of scavenger receptor which mediates the uptake of AcLDL in the lesion sites. The higher ^{125}I-CI in blood for this group of animals can be explained by their higher blood cholesterol levels (Table I). From Fig. 4, it was determined that the lesion areas which correlated with the autoradiography was about 5% of the total aorta. The specific radioactivity of the lesion sites of aorta was the highest among all the tissues taken, including liver and spleen (Table II). Thus we have shown that using AcLDL as a targeting carrier to atherosclerotic plaques achieves a high accumulation of ^{125}I-CI at these sites, as shown by the presence of the hottest spots in the lesioned areas among the major organs examined.

Sudan IV staining was carried out on the aortae in order to visualize the atherosclerotic lesions. The red lipid stain is an indication of lipid-laden foam cells, a hallmark of early atherosclerosis. As shown in Fig. 4, the lesion area corresponds to about 5% of the whole aorta.

The most severe lesion region was the ascending aorta at aortic arch. Blood flow through this section of aorta is turbulent due to the curve of the aortic arch and the three large branches. The highest incidence of human atherosclerosis is to be found in this region of the aorta. The hottest spots detected in the aortae of lesioned rabbits are strongly correlated to the location of highest incidence of human atherosclerosis.

Autoradiographs of aortae removed from both normal and atherosclerotic rabbits are shown in Fig. 4. The image is the result of accumulation of ^{125}I-CI/AcLDL. The area of the image superimposed with the staining result suggesting ^{125}I-CI/AcLDL has been taken up by the lesion site. Both Sudan IV and autoradiograph showed negative results with the aorta from control rabbits.

CONCLUSIONS

Our studies demonstrated that selective uptake and retention of ^{125}I-CI at the site of early atherosclerotic lesions can be achieved via the use of AcLDL as the carrier. This finding warrants further animal studies. We, therefore, plan to prepare ^{131}I- or ^{123}I-CI/AcLDL to be injected into rabbits having early atherosclerotic lesions. Imaging pictures of the rabbits will be acquired using gamma scintigraphy and will be assessed. These results are crucial before any human studies can be considered.

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REFERENCES

1. C. J. Schwartz, A. J. Valente, E. A. Sprague, J. L. Kelley, and R. M. Nerem. The pathogenesis of atherosclerosis, an overview. *Clin. Cardiol.* **14**:I-1–I-16 (1991).
2. E. N. Arnett, M. Isner, D. R. Redwood, K. M. Kent, W. P. Daker, H. Ackerstien, and W. C. Roberts. Coronary artery narrowing in coronary heart disease: Comparison of cineangiographic and necropsy findings. *Ann. Int. Med.* **91**:350–356 (1979).
3. Z. Vlodaver, R. Frech, R. A. Van Tassel, and J. E. Edwards. Correlation of the antimortem coronary arteriogram and the postmortem specimen. *Circulation* **47**:162–169 (1973).
4. R. G. Gerrity. The role of the monocyte in atherogenesis. I. Transition of blood-borne monocytes into foam cells in fatty lesions. *Am. J. Pathol.* **103**:181–190 (1981).
5. L. M. Buja, T. Kita, J. L. Goldstein, Y. Watanabe and M. S. Brown. Cellular pathology of progressive atherosclerosis in the WHHL rabbit. An animal model of familial hypercholesterolemia. *Arteriosclerosis* **3**:87–101 (1983)
6. J. L. Goldstein, Y. K. Ho, S. K. Basu and M. S. Brown. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA.* **76**:333–337 (1979).
7. S. Yla-Herttuala, M. E. Rosenfeld, S. Parthasarathy, E. Sigal, T. Sarkioja, J. L. Witztum, and D. Steinberg. Gene expression in macrophage-rich human atherosclerotic lesions. 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J. Clin. Invest.* **87**:1146–1152 (1991).
8. M. A. Longino, J. P. Weichert, S. W. Schwendner, S. M. Szabo, R. E. Counsell, and G. M. Glazer. Biodistribution of a new lipid-soluble CT contrast agent. Evaluation of cholesteryl iopanoate in the rabbit. *Invest. Radiol.* **18**:275–278 (1983).
9. V. N. Schumaker and D. L. Puppione. Sequential flotation ultracentrifugation. *Methods in Enzymology* **128**:155–171 (1986).
10. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72**:248 (1976).
11. S. K. Basu, J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholes-

terolemic fibroblasts. *Proc. Natl. Acad. Sci. USA* **73**:3178–3182 (1976).
12. R. H. Seevers, M. P. Groziak, J. P. Weichert, S. W. Schwendner, S. M. Szabo, M. A. Longino, and R. E. Counsell. Potential tumor- or organ-imaging agents. 23 Sterol esters of iopanoic acid. *J. Med. Chem.* **25**:1500–1503 (1982).
13. J. P. Weichert, M. A. Longino, D. A. Bakan, M. G. Spigarelli, T-S Chou, S. W. Schwendner, and R. E. Counsell. Polyiodinated triglyceride analogs as potential computed tomography imaging agents for the liver. *J. Med. Chem.* **38**:636–646 (1995).
14. M. R. DeGalan, S. W. Schwendner, R. W. S. Skinner, M. A. Longino, M. Gross, and R. E. Counsell. Iodine-125 cholesteryl iopanoate for measuring extent of atherosclerosis in rabbits. *J. Nucl. Med.* **29**:503–508 (1988).
15. A. D. Cardin, C. A. Price, N. Hirose, M. A. Krivanek, D. T. Blankenship, J. Chao, and S. J. T. Mao. Structural organization of apolipoprotein B-100 of human plasma low density lipoproteins. Comparison to B-48 of chylomicrons and very low density lipoproteins. *J. Biol. Chem.* **261**:16,744–16,748 (1986).
16. M. Samadi-Baboli, G. Favre, P. Canal, and G. Soula. Low density lipoprotein for cytotoxic drug targeting: improved activity of elliptinium derivative against B16 melanoma in mice. *Br. J. Cancer* **68**:319–326 (1993).
17. H. Sinzinger, H. Bergmann, J. Kaliman, and P. Angelberger. Imaging of human atherosclerotic lesions using ^{123}I-low density lipoprotein. *Eur. J. Nucl. Med.* **12**:291–292 (1986).
18. H. Sinzinger and P. Angelberger. Imaging and kinetic studies with radiolabeled autologous low-density-lipoproteins (LDL) in human atherosclerosis. *Nucl. Med. Comm.* **9**:859–866 (1988).
19. A. B. Roberts, A. M. Lees, R. S. Lees, H. W. Strauss, J. T. Fallon, J. Taveras, and S. Kopiwoda. Selective accumulation of low density lipoproteins in damaged arterial wall. *J. Lipid Res.* **24**:1160–1167 (1983).
20. J. M. Rosen, S. P. Butler, G. E. Meinken, T. S. T. Wang, R. Ramakrishan, S. C. Srivastava, P. O. Alderson, and H. N. Ginsberg. Indium-111 labeled LDL: A potential agent for imaging atherosclerotic disease and lipoprotein distribution. *J. Nucl. Med.* **31**:343–350 (1990).
21. J. M. Nicolas, B. Leclef, H. Jardez, A. Keyeux, J A. Melin, and A. Trouet. Imaging atherosclerotic lesions with ^{111}In-labeled low density lipoprotein. (abstract) Vienna. *Proceedings of the International Atherosclerosis Congress*. p A219 (1989).
22. R. S. Lees, H. D. Garabedian, A. M. Lees, D. J. Schumacher, A. Miller, J. L. Isaacsohn, A. Derksen, and H. W. Strauss. Technetium-99m low density lipoprotein: Preparation and biodistribution. *J. Nucl. Med.* **26**:1056–1062 (1985).
23. A. M. Lees, R. S. Lees, F. J. Schoen, J. L. Issacsohn, A. J. Fischman, K. A. McKusick, and H. W. Strauss. Imaging human atherosclerosis with^{99m} Tc-labeled low density lipoprotein. *Atherosclerosis* **8**:461–470 (1988).
24. D. E. Atsma, R. I. J. Feitsma, J. Camps, F. M. van't Hooft, E. E. van der Wall, W. Nieuwenhuizen, and E. K. J. Pauwels. Potential of^{99m}Tc-LDLs labeled by two different methods for scintigraphic detection of experimental atherosclerosis in rabbits. *Arteriosclerosis Thrombosis* **13**:78–83 (1993).
25. I. Kenji, K. Toru, K. Noriaki, N. Yutaka, and K. Chuichi. Uptake of acetylated LDL by peritoneal macrophages obtained from normal and Watanabe heritable hyperlipidemic rabbits, an animal model for familial hypercholesterolemia. *Biochim. Biophys. Acta.* **962**:387–389 (1988).
26. M. S. Brown and J. L. Goldstein. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Review Biochem.* **52**:223–261 (1983).
27. J. Narula, A. Petrov, K. Y. Pak, C. Ditlow, F. Chen, and B. A. Khaw. Noninvasive detection of atherosclerotic lesions by^{99m} Tc-based immunoscintigraphic targeting of proliferating smooth muscle cells. *Chest* **111**:1684–1690 (1997).
28. B. A. Allison, M. T. Crespo, A. K. Jain, A. M. Richter, Y. N. Hsiang, and J. G. Levy. Delivery of benzoporphyrin derivative, a photosensitizer, into atherosclerotic plaque of Watanabe heritable hyperlipidemic rabbits and balloon-injured New Zealand rabbits. *Photochem. Photobiol.* **65**:877–883 (1997).