PRELIMINARY NOTE

External Imaging of Human Atherosclerosis

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Autologous plasma low-density lipoproteins labeled with I-125 were used as a tracer to identify atherosclerotic lesions in the carotid arteries of the neck. Following intravenous injection of I-125-LDL, images were made at intervals from 6 to 36 hr with the gamma camera in three patients with known carotid disease and one control subject. The carotid lesions, confirmed by angiography, were imaged successfully in all three patients, whereas no focal LDL accumulation was visible in the carotid arteries of the control subject. The findings suggest that it may be possible to image atherosclerosis externally and thus to follow the course of the disease.

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Atherosclerosis is a chronic, progressive disease of blood vessels, clinically silent until late in its course. Diagnosis ordinarily depends on the detection of impaired blood flow or decreased blood pressure distal to an arterial narrowing (*1*). In contrast to the relative hemodynamic silence of the disease until late in its course, metabolic changes in the arterial wall are common even in the early phases of atherosclerosis (*2–4*). These changes are characterized not only by cellular proliferation in the arterial wall (*2,4*), but also by accumulation in the wall of beta or low-density lipoproteins (LDL), the major cholesterol-carrying proteins in the blood (*3,5*). To determine whether the metabolic activity in diseased areas of the arterial wall might permit sufficient concentration of LDL in the damaged arterial wall to allow detection of the lesions by gamma imaging, a pilot study of four human subjects was performed using I-125-labeled LDL as the radiopharmaceutical.

METHODS

Patients. Three subjects with known atherosclerosis involving the carotid arteries, and one hypercholesterolemic subject without carotid disease, were studied. Informed written consent was obtained from each patient before the studies were performed. Their clinical status is summarized in Table 1. Three patients had angiographically documented carotid atherosclerosis, while the fourth subject had no carotid bruits and a normal carotid duplex Doppler ultrasound examination (*6*). To block thyroid uptake of I-125, Subject I received 5 mg of potassium iodide solution, and Subjects 2–4 received 50 mg daily, beginning two days before and continuing for 14 consecutive days after tracer administration. All aspects of the study were approved by the Human Studies, Radioisotope, and Pharmacy Committees of both MIT and MGH.

Lipoprotein preparation. Each patient was requested to refrain from eating after 9 p.m. the evening before the study was begun. The next morning at 9 a.m., 100 ml of the patient's blood was drawn into sterile tubes containing disodium EDTA (1 mg/ml of blood), and the plasma was separated at 4°C. Ultracentrifugation and all subsequent steps were carried out under sterile conditions. LDL was isolated between densities of 1.025–1.050 g/ml (*7*), and was dialyzed against normal saline, pH 8.6, for at least 4 hr and iodinated with $Na^{125}I$ by a modification of the MacFarlane method (*7*). The I-125-LDL was dialyzed overnight against 500 volumes of normal saline, with one change. Labeling efficiency was 25–35% and the protein was >98% precipitable with trichloroacetic acid. One hundred microcuries of autologous I-125-LDL (1–2 mg protein), diluted with 1.5 volumes of sterile 5% human albumin, were injected into the antecubital vein of each subject.

External imaging. A scintillation camera with com-

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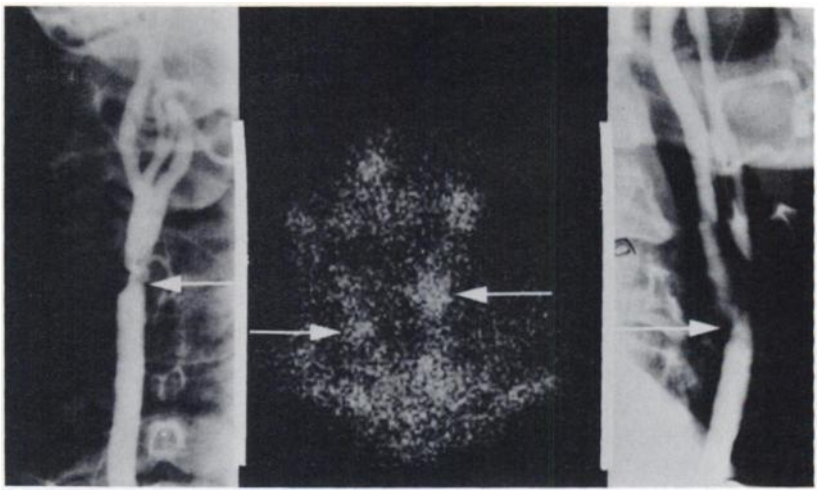

Fig. 1. Comparison of bilateral carotid angiograms with a 2-day scintigram from Subject 2. Thyroid gland is visible at bottom of scintigram, and the blood pool in cerebral venous sinuses at top. The isolated right common carotid lesion (left side of scintigram) sequesters I-125-LDL, while extensive disease of left common and internal carotids, which extends much higher in the neck, accumulates far more radiolabeled lipoprotein (right side of scintigram).

puter for data storage and display were used to image each patient at six hours, one day, and two days after I-125-LDL injection. Photons from the I-125 were detected with a baseline of 20 KeV and an upper level of 50 KeV. Images of the neck were recorded with a parallel-hole low-energy collimator in the anterior or both lateral views, for 10 min each at 6 hr after injection, and for 20 min each at one and two days. In one patient, the renal-adrenal region was imaged in the posterior view for 20 min at two days after injection. Approximately 50,000 counts were collected in the 6-hr images, and 20K–30K counts at one and two days. An aliquot of the I-125-LDL to be injected was diluted 1:100 and imaged in a thyroid neck phantom* to permit gross quantitation of the imaged activity in the neck.

RESULTS

At 6 hr after injection, the blood pool in the vessels of the neck was visible in all subjects, and no definite areas of localized LDL accumulation were observed. At one day blood-pool activity had decreased and focal I-125 LDL uptake began to appear. By two days, discrete LDL uptake was observed in the carotid arteries of two of the three patients with known carotid disease; an image strongly suggestive of LDL uptake was seen in the third patient. No focal I-125-LDL accumulation was seen in the vertebral arteries. Attempts to visualize the coronary and renal vessels and the adrenal glands were unsuccessful.

The anterior image of Subject 2 is shown in Fig. 1. There were two focal areas of I-125-LDL uptake in the carotid arteries; these corresponded to the angiographic locations of the lesions. In the angiogram of the right carotid system, atherosclerosis was present mostly below the bifurcation, whereas on the left side there was some involvement of the distal common carotid, but most of the disease was above the bifurcation in the internal carotid artery. Both the right and left carotids had focal uptake of I-125-LDL, with the area on the right at a lower level than that on the left. No focal LDL uptake was seen in regions that did not appear diseased on the arteriogram. The images of subject 3 were consistent with focal LDL accumulation at both carotid bifurcations, but uptake in the thyroid and the low number of total counts made interpretation difficult.

No areas of focal LDL accumulation could be detected in the control subject, who had no evidence of carotid disease. The concentrations of I-125-LDL in areas of focal accumulation was between 0.01 and 0.1% of the administered dose, and was maximal at 30–48 hr; this activity was 1.5 to 3 times the concentration in the surrounding vessels. The thyroid activity was less than 0.5% of the administered dose.

DISCUSSION

These four studies demonstrate the possibility of imaging atherosclerotic disease in the carotid arteries, even with a nuclide that is far from optimal in terms of photon energy. Radiolabeled LDL was chosen as the imaging agent because earlier work with rabbits had demonstrated that LDL was actively and preferentially taken up by the leading edge of regenerating endothelium in rabbit aorta that had been de-endothelialized with a balloon catheter (*8*). The LDL was concentrated at the healing edge as much as 16-fold in comparison with the surrounding healed artery, and on autoradiography was localized in the thickened intima and media underneath the healing endothelium.

Human atherosclerosis is believed to involve focal de-endothelialization by a variety of mechanisms, which is followed by endothelial regeneration (*4*). Thus focal uptake of I-125-LDL was expected where active atherosclerotic disease was occurring. Conversely, where endothelial regeneration was not occurring, no LDL accumulation was expected.

Additional human studies will be necessary to define the exact relationship between LDL uptake by the arterial wall and the images produced. In our patients, the

lesions were not clearly defined in the early images. The improved visualization on the later images may have resulted either from a decrease in the blood concentration of I-125-LDL, or an increase in lesion concentration, or both.

Iodine-125 was chosen because I-125 LDL is a well-characterized radiopharmaceutical that has been administered to patients for metabolic studies. The plasma half-life of I-125 LDL in man is about 2½ days in normal subjects, longer in hypercholesterolemia (9–12). The labelled protein is degraded intracellularly largely to iodotyrosine and eventually is excreted as such in the urine. Since a dose of only 100 μCi was used for these studies—and even then a maximum of only 50,000 counts were recorded—the images took a long time to acquire. In addition, the low-energy photon of I-125 results in very poor spatial resolution in the images. Nevertheless, focal LDL uptake was observed in the carotid vessels of three patients, and no uptake in the control subject.

The pathologic studies of Hoff (*5*) showed that human atherosclerotic lesions contain bound LDL in relatively high concentration. Our imaging studies suggest that there is significant entry of LDL into the damaged arterial wall and that LDL entry is relatively rapid. These data are also consistent with our animal studies, which showed that the moiety accumulating in the arterial wall is macromolecular LDL (*8*).

The results of this preliminary investigation suggest that LDL conjugation with a gamma emitter having better imaging characteristics, such as I-123, or Tc-99m, may be worthwhile, since it appears that active atheromatous lesions can be imaged with radiolabeled LDL. If images of good quality could be obtained using a better emitter, the goal of easily following progression and regression of atherosclerosis might be realized.

FOOTNOTE

*Picker.

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