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Report

Radioiodinated Cholesteryl Iopanoate as a Potential Probe for the *in Vivo* Visualization of Atherosclerotic Lesions in Animals

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Radioiodinated cholesteryl iopanoate, a nonhydrolyzable cholesteryl ester probe, showed increased uptake into atherosclerotic aortas of cholesterol-fed rabbits in comparison with normal rabbits. Autoradiography of the aortas showed the radioactivity to be concentrated in areas of visible atherosclerotic involvement. Lipid extraction and thin-layer chromatography of this tissue as well as liver, adrenal, and plasma confirmed the resistance of this probe to hydrolysis. These findings suggest that ^{125}I-cholesteryl iopanoate may prove useful for noninvasively monitoring atherosclerosis in intact laboratory animals.

KEY WORDS: radioiodinated cholesteryl iopanoate; nonhydrolyzable cholesteryl ester probe; atherosclerosis; rabbit.

INTRODUCTION

Although interest in developing antiatherogenic agents has increased rapidly in the past several years, these efforts have been hampered by a lack of a suitable animal model (1). Several species have been found to be susceptible to the development of atherosclerosis with the proper dietary regiman, but a noninvasive means of assessing the severity of this disease is noticeably lacking. One of the efforts of this laboratory, therefore, has been to develop a radioiodinated probe for ascertaining the progression or regression of atherosclerosis noninvasively in the intact animal.

The prominent lipids in atherosclerotic lesions are free and esterified cholesterol. Even though arterial smooth muscle cells are capable of synthesizing cholesterol de novo, the major portion which accumulates during atherosclerosis is derived from serum lipoproteins (2–4). However, a portion of the cholesteryl esters taken up in this manner is subsequently hydrolyzed and excreted from the cells of the artery into the bloodstream as free cholesterol. This equilibrium between free and esterified cholesterol reduces the ability of radiolabeled natural cholesteryl esters to accumulate at the sites of injury. Although probes such as tritiated cholesteryl linoleyl ether, a nondegradable analogue of cholesteryl linoleate developed by Stein *et al.* (2), have proved useful for measuring influx rates of natural cholesteryl esters into atherosclerotic lesions of rabbits following postmortem analysis, the weak beta radiation associated with tritium prevents the use of such a probe for imaging lesions in the intact animal. To overcome this problem, studies in our laboratory have focused on the possibility of employing a radioiodinated derivative of cholesteryl ester for such purposes.

Recent reports from our laboratory have outlined the synthesis of a variety of cholesteryl ester analogues wherein the acyl moiety serves as a carrier for radioiodine (5–7). *In vivo* studies in rats and rabbits revealed that several of these esters were resistant to *in vivo* hydrolysis (7,8). This property suggested their use as possible probes for monitoring cholesteryl ester accumulation in atherosclerotic lesions by such techniques as gamma-camera scintigraphy. An ester of cholesterol and iopanoic acid (Telepaque), a widely used cholecystographic agent, appeared to be particularly promising for this purpose. This report describes the tissue distribution of ^{125}I-cholesteryl iopanoate (^{125}I-CI) in normal New Zealand White (NZW) rabbits and those made atherosclerotic by balloon-catheter endothelial injury to the aorta and a 2% cholesterol diet.

MATERIALS AND METHODS

Male NZW rabbits were obtained from Langshaw Farms, Augusta, Mich., and averaged 3.6 kg at sacrifice. These animals were divided into control (N = 9) and atherosclerotic (N = 14) groups. Atherosclerosis was induced by producing endothelial injury to the aorta using a 3F Fogarty embolectomy balloon catheter introduced via the femoral artery (9). Flocillin (300,000 U) was administered im 1 day prior to and 1 day after surgery. Anesthesia was induced using xylazine (9 mg/kg) and ketamine (50 mg/kg) im. The balloon was inflated with 0.5 ml of air and passed three or four times through the entire aorta. The femoral artery was ligated at the end of this procedure. The atherosclerotic group was fed 2% cholesterol-enriched chow (Purina Special Diets Plant, Richmond Ind.) and water ad libitum for 6 weeks. Control rabbits underwent no surgery and were fed a normal diet.

^{125}I-CI (average specific activity, 150.8 μCi/mg) was prepared and formulated in saline containing 2% Tween-20

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as described previously (7). Radiochemical purity was determined before and after formulation by thin-layer chromatography (TLC) using silica-gel plates with fluorescent indicator (Eastman Kodak, Rochester, N.Y.) developed in benzene:ethyl acetate (9:1, v/v). TLC plates were visualized under ultraviolet (UV) light.

Six weeks after surgery, ^{125}I-CI was administered via the ear vein to control and atherosclerotic rabbits. The average dose was 31.4 μCi/kg. Control and atherosclerotic animals were each divided into three groups, which were sacrificed at 24, 48, and 72 hr, respectively. The animals were sacrificed by cardiac puncture while under pentobarbital anesthesia. Various tissues were excised and assayed for radioactivity with a gamma counter (Searle 1185 gamma-spectrometer) and expressed as the percentage administered (kg) dose per gram (μCi/mg tissue divided by the administered dose multiplied by the body weight). Bile was collected by syringe, weighed, and counted as above. The counting efficiency of the gamma-spectrometer was 82%.

The aortas were removed at sacrifice, trimmed of adventitial fat, opened longitudinally, and rinsed with saline to remove traces of blood. These aortic strips were blotted dry, mounted on cardboard, wrapped in plastic wrap, and exposed to Kodak XAR-5 film for 40 hr at −70°C. Some aortas were also treated with oil red O lipid stain before photography. After autoradiography, the aortas were rehydrated with saline, blotted, and cut into 1-cm sections. These sections were then weighed and counted for radioactivity.

To test the stability of ^{125}I-CI *in vivo,* samples of liver, adrenal, and plasma from both normal and atherosclerotic rabbits were extracted using chloroform/methanol as described by Folch *et al.* (10). Samples of aorta from atherosclerotic rabbits were also extracted. Because of the extremely low levels of radioactivity found in the aorta from normal rabbits, however, lipid extraction was not performed in this instance. The organic phase was analyzed by TLC to determine the presence of intact ^{125}I-CI or its metabolites. TLC plates were scanned using a radiochromatogram scanner (Berthold Model 6000) and then cut into 1-cm sections for gamma counting.

RESULTS

At 24 hr following the administration of ^{125}I-CI to normal rabbits, the adrenals and liver were among those tissues found to contain high concentrations of radioactivity. As shown in Fig. 1, the concentration of radioactivity increased with time in the adrenals but remained relatively constant in the liver. The spleen was the only other tissue to retain high levels of radioactivity at 24 hr [3.70 ± 0.70% administered (kg) dose/g], and this activity declined with time. Much lower levels of radioactivity were apparent in these tissues in the atherosclerotic rabbits. For example, at 24 hr the concentration of radioactivity in the adrenals was essentially one-half the normal value, whereas that in the liver and spleen was approximately one-third the normal concentration.

The reverse was true for blood and aorta. As shown in Fig. 2, the concentration of radioactivity in blood and aortas of the atherosclerotic rabbits at 24 hr (N = 5) was approximately 3-fold and 40-fold the concentrations in normal rabbits (N = 3), respectively. Although blood radioactivity

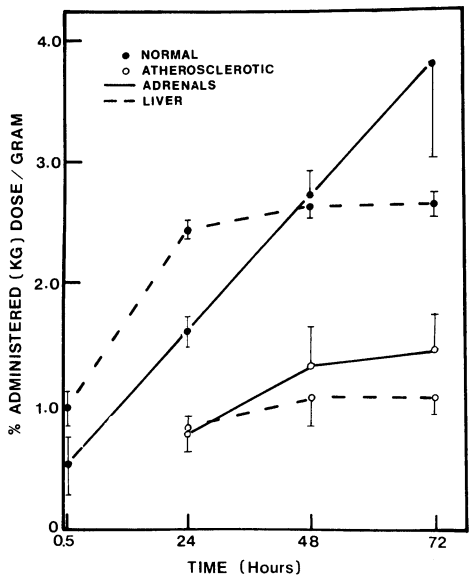

Fig. 1. Radioactivity recovered in the adrenals and livers of atherosclerotic (○) or normal (●) rabbits (N = 3–5) between 0.5 and 72 hr following intravenous administration of ^{125}I-CI.

was seen to decline slowly with time in the atherosclerotic rabbits, the radioactivity in the aorta remained relatively constant, such that the blood/aorta ratio at 24 hr was 3.41 (0.645/0.189).

Although not shown in Fig. 2, atherosclerotic rabbits sacrificed at 6 (N = 4) and 14 (N = 2) days after the administration of ^{125}I-CI showed blood/aorta ratios of 0.72 (0.188/0.265) and 0.42 (0.068/0.162), respectively, which further illustrates the retention of radioactivity by the aorta.

Autoradiograms of aortas from normal and atherosclerotic rabbits (Fig. 3) also demonstrated a much higher concentration of radioactivity in the atherosclerotic vessels. Moreover, the areas of intense darkness on the autoradiograms corresponded to visibly atheromatous regions as well as to those areas most intensely colored with oil red O lipid stain. In addition, radioactivity present in the aorta, as de-

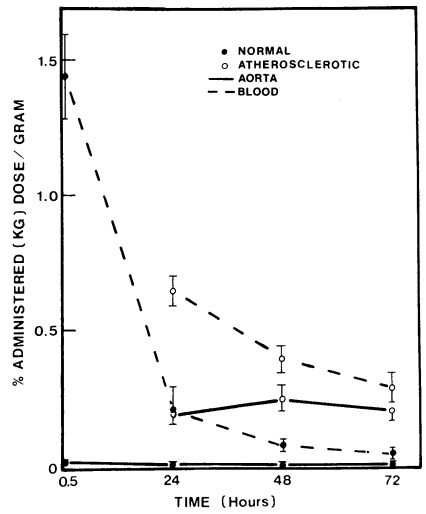

Fig. 2. Radioactivity recovered in aortas and blood of atherosclerotic (○) or normal (●) rabbits (N = 3–5) between 0.5 and 72 hr following intravenous administration of ^{125}I-CI.

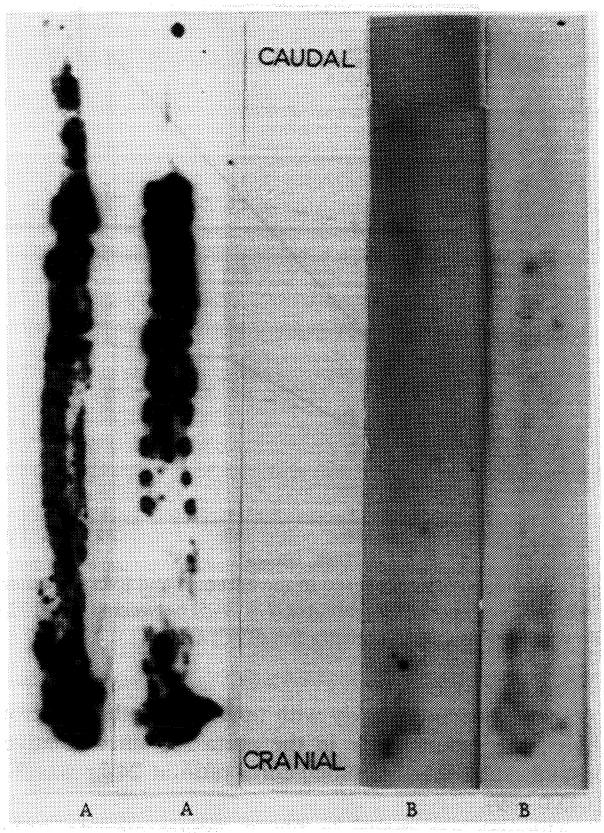

Fig. 3. En-face autoradiographs of whole aortas of either atherosclerotic (A) or normal (B) rabbits injected with ^{125}I-CI and sacrificed 48 hr later. The probe was injected 6 weeks after denudation of the aorta in atherosclerotic rabbits.

termined by counting 1-cm sections, was found to correlate with those regions visibly displaying the most atherosclerosis.

Extraction of the above tissues with chloroform/methanol indicated that over 80% of the radioactivity was extractable into the organic phase (Table I). Moreover, subsequent TLC analysis of this phase revealed that over 80% of the radioactivity comigrated with a cholesteryl iopanoate standard. There was very little difference between values obtained from normal and those obtained from atherosclerotic rabbits. Although Table I shows values for animals sacrificed at 72 hr, similar results were obtained with rabbits sacrificed at earlier time periods.

DISCUSSION

In this report we have outlined some properties of ^{125}I-cholesteryl iopanoate, a radioiodinated cholesteryl ester analogue that appears in this and other *in vivo* studies (7,8) to be resistant to *in vivo* hydrolysis. The resistance of ^{125}I-CI to hydrolysis was based upon two observations in this study: (i) lipid extraction of the liver, adrenals, and plasma of both normal and atherosclerotic rabbits and thin-layer chromatography of the resulting extracts showed a majority of the radioactivity to be present as parent compound 72 hr after administration; and (ii) the bile contained very little radioactivity, a finding which would be unexpected if ipoanoic acid was liberated by ester hydrolysis.

Both the liver and the adrenals contained more radiolabel per gram of tissue in the control animals than the hypercholesteremic animals (Fig. 1). This is consistent with the observation that both of these tissues have high densities of low-density lipoprotein (LDL) receptors that become down-regulated during cholesterol feeding and thereby accumulate reduced levels of intracellular cholesteryl ester (11,12). Moreover, similar to other radioiodinated cholesteryl esters (7,8,13,14), we have found that ^{125}I-CI rapidly becomes associated with plasma lipoproteins soon after iv administration (data not presented). This supports the notion that ^{125}I-CI is taken up into cells by lipoprotein-mediated processes similarly to naturally occurring cholesteryl esters.

Both total plasma cholesterol and total plasma lipoprotein levels are known to increase dramatically in NZW rabbits fed a cholesterol-enriched diet for 10 days or more (15). This results in a dilution of the plasma lipoprotein concentration of ^{125}I-CI in the atherosclerotic versus the normal rabbits. This can account, at least partially, for the observed decreased plasma clearance of ^{125}I-CI in the atherosclerotic group. Thus, the plasma half-life of ^{125}I-CI in the hypercholesterolemic rabbits was found to be approximately 42 hr, as opposed to approximately 22 hr in the normal rabbits.

Autoradiograms of the aortas showed increased uptake of ^{125}I-CI in atherosclerotic rabbits compared to controls. Furthermore, the darkest regions of the films matched with areas of atherosclerotic involvement (Fig. 3), and the measured fraction of the dose reaching the tissue confirmed this observation. At 48 hr the ratio of atherosclerotic to normal aorta radioactivity was 44.9, a value that remained relatively unchanged for at least 6 days after administration.

These findings are encouraging, for they indicate that

Table I. Results of Lipid Extraction of Tissues from Normal and Atherosclerotic Rabbits at 72 Hours[a]

	Normal (N = 3)		Atherosclerotic (N = 1)	
Tissue	% radioactivity in organic phase	% radioactivity comigrating with CI	% radioactivity in organic phase	% radioactivity comigrating with CI
Adrenal	83.0 ± 2.6	92.3 ± 0.5	87.1	92.0
Aorta	—	—	94.9	82.9
Liver	93.5 ± 0.6	85.4 ± 2.0	86.9	81.4
Plasma	82.1 ± 3.5	80.0 ± 4.5	83.5	89.3

[a] Atherosclerotic rabbit was given 2% cholesterol-enriched chow for 8 weeks following endothelial denudation and was then intravenously administered ^{125}I-CI 72 hr prior to sacrifice.

^{125}I-CI may be a useful tool for determining the requirements for delivering diagnostic agents specifically to atherosclerotic lesions. Such agents labeled with gamma-emitting isotopes would offer a method for noninvasively monitoring the progression of atherosclerosis in pharmacologically treated or untreated hyperlipidemic animals. Efforts to image externally atherosclerosis in the abdominal aorta in rabbits using ^{125}I-CI have recently been concluded and will be reported at a latter date.

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