Noninvasive Assessment of Lipid Disposition in Treated and Untreated Atherosclerotic Rabbits

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In an effort to visualize whole body cholesteryl ester (CE) deposition using the nuclear medicine imaging technique of gamma camera scintigraphy, 125I-cholesteryl iopanoate (125I-CI), a nonhydrolyzable CE analogue, was used as a marker for CE deposition in atherosclerotic New Zealand white rabbits. Groups of animals were fed either a cholesterol-enriched diet (2%, w/w) or the same diet supplemented with the hypolipidemic drugs colestipol (1%, w/w) and/or clofibrate (0.3%, w/w). Injections of 125I-CI were administered biweekly. At the end of 15 weeks, animals were scintigraphically scanned and sacrificed for tissue analysis. The results demonstrated that while drug treatment had no significant effect on plasma lipid levels, it substantially lessened atherosclerotic involvement in the thoracic-abdominal aorta. These differences in aortic lipid accumulation were reflected in the whole-body scans which showed a reduction in tissue accumulation of 125I-CI in the drug-treated groups. Gamma camera scintigraphy thus represents a rapid means of visualizing tissue CE accumulation which could facilitate the evaluation of lipid-lowering drug efficacy and possible antiatherosclerotic effect.

KEY WORDS: colestipol; clofibrate; noninvasive imaging; atherosclerosis; rabbit; nonhydrolyzable cholesteryl ester.

INTRODUCTION

Hypercholesteremia is a primary risk factor for atherosclerosis, which can lead to coronary heart disease and cerebrovascular insufficiency. In an effort to reduce the morbidity and mortality associated with the development of advanced lesions, several different classes of lipid-lowering drugs have been developed. These drugs have been shown to reduce the plasma levels of lipids and lipoproteins through one or more of a variety of mechanisms (1). In addition, a number of other agents such as estrogen (2), chlorpromazine (3), calcium antagonists (4,5), β-blockers (6), and anti-inflammatory agents (7) have been shown to be beneficial in reducing atherogenesis in animal models without significantly affecting plasma lipid levels.

Because of the complexity of actions of various drugs, drug combinations, and agents of unknown potential in reducing atherogenesis, a simple means of evaluating an effect on whole body cholesterol disposition would be valuable in assessing drug efficacy. One potential approach under investigation in our laboratory involves the use of gamma camera scintigraphy, a nuclear medicine imaging technique, in the extracorporeal detection of radioactivity in animals injected with a radiolabeled cholesteryl ester (CE) derivative. This procedure necessitates the use of a CE derivative radiolabeled with a gamma-emitting isotope such as 125I, 131I, or 99mTc. 125I-Cholesteryl iopanoate (125I-CI), a CE analogue that has been used in several studies in our laboratory, is well suited for studies of this nature because the ester linkage is resistant to hydrolysis (8,9), thus allowing the compound to accumulate in tissues once taken up. Moreover, as compared to other more commonly used methods of assessing the tissue deposition of radioactivity, scintigraphic imaging permits the acquisition of images showing the whole-body distribution of radioactivity within a relatively short period of time. In addition, sacrifice of the animal is not necessary, an important consideration when using larger, more expensive animals.

Using 125I-CI as a marker for CE, the dual purpose of this study was to determine whether the hypolipidemic drugs colestipol and clofibrate could slow the progression of atherosclerosis in cholesterol-fed New Zealand white rabbits and, more importantly, to determine whether differences in whole-body accumulation of 125I-CI could be detected in the treated and untreated groups using gamma camera scintigraphy.
MATERIALS AND METHODS

Preparation of \(^{125}\text{I}-\text{Cholesteryl Iopanoate}\)

\(^{125}\text{I}-\text{CI}\) was synthesized and radiiodinated as previously described (8) and had an average specific activity of 0.19 Ci/mmol. Radiochemical purity was checked by thin-layer chromatography (TLC) using Eastman silica gel chromatographic sheets developed in a hexane/ethyl acetate (5:2) solvent system. The TLC strips were then scanned with a Berthold 6000 radiochromatogram scanner. The compound was formulated for administration to animals in a Tween-20/physiological saline vehicle with the Tween constituting <3% of the total volume. Radiochemical purity of the formulated compound was assessed prior to injection as described above.

Study Design

Female New Zealand white rabbits (Shankin’s Rabbitry, Warren, MI), weighing 2.6 ± 0.3 kg (mean ± SD) at the beginning of the study, were divided into five treatment groups. One group was fed normal rabbit chow (N; n = 3), and the other four groups (n = 6 in each group to start) were fed chow containing 2% cholesterol (Purina Test Diets, Richmond, IN). Of these animals, one group remained untreated (C; n = 5 at the completion of the study), and the others received diets supplemented with colestipol [CP; 1% (w/w) in diet; n = 5 upon completion], clofibrate [CF; 0.3% (w/w) in diet; n = 3 upon completion], or a combination of the two drugs (CPCR; n = 6 upon completion). Five animals were euthanized due to broken backs which occurred during handling (n = 2), ear infections (n = 2), and pneumonia (n = 1). The colestipol was obtained from the Upjohn Company (Kalamazoo, MI), and the clofibrate was made from commercially available clofibric acid by esterification with ethanol according to the reported procedure (10). The rabbits were given 100 g of the designated diet daily over a 15-week period, and its consumption was monitored.

Beginning 1 week after the start of the diet regimens, animals were injected twice weekly via the marginal ear vein with 10 µCi of \(^{125}\text{I}-\text{CI}\) (solubilized in saline as described above) in a volume of approximately 0.2 ml. The last injection was given 13 days prior to the end of the study to allow the \(^{125}\text{I}-\text{CI}\) to be cleared from the blood.

Fifteen weeks after the start of the study, animals were anesthetized with intramuscular injections of xylazine (8 mg/kg) and ketamine (50 mg/kg) and scintigraphically scanned using a gamma camera (Ohio Nuclear Mobile Camera) with a high-sensitivity collimator. Animals were placed in a prone position with the camera centered over the back such that liver radioactivity was visible in the top portion of the resulting scan. Acquisition time for all \(^{125}\text{I}-\text{CI}\) scans was 30 min. A few animals were also injected with \(^{99m}\text{Tc}\)-sulfur colloid in order to visualize the bone marrow. Animals were then sacrificed by exsanguination via cardiac puncture under sodium pentobarbital anesthesia, and blood and tissues (including aorta, adrenal, liver, thyroid, bile, and gall bladder) were removed for analysis.

Tissue Analysis

Blood was collected into Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at low speed to obtain plasma. Using enzyme assay kits, the plasma was analyzed for total cholesterol (CooperBiomedical, Diagnostic Division, Freehold, NJ) and triglyceride (Sigma Diagnostics, St. Louis, MO).

In addition, the aortas (from the arch to the femoral bifurcation) were removed, rinsed in saline, and dissected free of fat and connective tissue. The arteries were then opened longitudinally and covered with clear plastic wrap, and the perimeter, lesions, and ostia were traced onto plastic sheets. The area involved in lesions was subsequently determined by planimetry. The aortas were cut into 1-cm segments and counted for radioactivity in a Searle 1185 gamma counter (counting efficiency approximately 87%). These sections were then homogenized, and lipid extractions (11) were performed on the pooled aortic sections constituting the arch (the first 2 cm of the vessel) and the thoracic-abdominal aorta of each animal. The total and free cholesterol contents of these extracts were determined using a fluorometric assay (12).

In order to ascertain the stability of the \(^{125}\text{I}-\text{CI}\), total lipid was extracted from samples of plasma, liver, and adrenal into chloroform-methanol (2:1) (11). The percentage radioactivity recovered in the organic phase was calculated, and the percentage of this material which comigrated with a \(^{125}\text{I}-\text{CI}\) standard was determined by TLC analysis in a hexane/ethyl acetate (5:2) solvent system as described above.

RESULTS

The weights of the animals as well as the daily chow consumption of each animal were monitored throughout the study. Although the weights of all groups of animals were similar at the outset, the animals in the cholesterol-fed groups were heavier than the N-group animals at the end of the study [3.4 ± 0.2 kg versus 3.0 ± 0.2 kg, respectively (mean ± SD)]. The chow consumption was not significantly different among any of the groups and averaged 98 g/day/rabbit.

The plasma cholesterol and triglyceride levels were markedly elevated in all animals receiving the cholesterol-supplemented diets (Fig. 1). Triglyceride levels were two to three times that of group N, and the cholesterol levels were 25- to 30-fold increased. As compared to group C, however, the drug treatments did not significantly alter these parameters.

The atherosclerotic involvement of the aortas was assessed by measuring the percentage surface area involved in lesions, the cholesteryl ester content of the artery, and the aortic accumulation of radioactivity [expressed as percentage injected dose (adjusted for decay) per gram of tissue multiplied by the kilogram weight of the animal]. The results are separated into values for the arch (Fig. 2) and the remainder of the aorta, the thoracic and abdominal regions (Fig. 3). This division was made since the first 2 cm of the aorta was observed to be almost uniformly atherosclerotic in all the cholesterol-fed rabbits, a finding consistent with the greater permeability of this region of the vessel to blood-borne substances such as Evans blue dye, albumin, and cholesterol (13). As compared to group N, the arch of all cho-