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IN VIVO METABOLISM OF I-123 LABELED
SEMISYNTHETIC LOW DENSITY LIPOPROTEINS

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I. ABSTRACT

We previously observed that small model beta-strand peptides (MBPs) selectively bind to human low density lipoprotein (hLDL) in vitro, and that some MBPs can be labeled with I-123-tyramine cellobiose (I-123-TyC). For this project we proposed to prepare radioiodinated "semisynthetic" hLDL by adsorption of prelabeled I-123-TyC-MBPs to the native hLDL particle surface, as a rapid and convenient alternative to covalent synthesis of I-123-TyC-hLDL. We hypothesized that metabolism of semisynthetic hLDL should mimic that of covalently labeled native hLDL, and we originally planned (i) to evaluate the biodistribution in rabbits of semisynthetic hLDL; (ii) to determine effects of prior oxidation and acetylation of the adsorbing hLDL on binding of MBPs and upon biodistribution of semisynthetic particles; and (iii) to begin biodistribution studies with semisynthetic hLDL in human subjects, with the eventual goal of application to experimental and clinical nuclear imaging studies.

In the course of this work we synthesized a radioiodotyrosine-containing MBP, designated betaY, as a more suitable adsorbant to hLDL than radioiodine-TyC-MBP, and optimized conditions for preparing radioiodine-betaY:hLDL. In rabbits both betaY and betaY:hLDL complexes were cleared from the bloodstream much more rapidly than radioiodine-TyC-hLDL or In-111-hLDL, and betaY in either form showed a biodistribution pattern different from that of directly radiolabeled hLDL. Even though radioiodine-betaY can be quickly and easily produced, we conclude that neither betaY alone nor semisynthetic betaY:hLDL particles are likely to prove useful as tracers of hLDL metabolism in vivo.

II. PROGRESS REPORT

A. Initial studies with semisynthetic hLDL.

In document DOE/ER/60614-1 (final technical report for agreement DE-FG02-87ER60614) we reported the successful labeling of three amphiphilic MBPs with radioiodine-TyC. These included the tridecapeptide $\text{NH}_2\text{-(Val-Ser-Val-Lys)}_3\text{-Val-COOH}$, which we labeled with I-125-TyC at about 40% efficiency and with I-123-TyC at about 10-20% efficiency. After incubation of labeled tridecapeptide with hLDL, semisynthetic I-123-TyC-MBP:hLDL particles were separated from reactants by gel filtration.

We conducted a limited biodistribution survey in rabbits of the labeling intermediates unreacted I-123, unconjugated I-123-TyC, and I-123-TyC-MBP as well as of I-123-TyC-MBP:hLDL. As expected, unreacted I-123 (as NaI) was taken up by the thyroid and excreted in the urine. Unconjugated I-123-TyC also appeared rapidly in the urine, but did not appreciably enter the thyroid. To our surprise, both I-123-TyC-MBP (injected without prior adsorption to hLDL), and the complex of I-123-TyC-MBP:hLDL were rapidly converted to hepatic radioactivity, and within minutes thereafter, to activity within the small intestinal lumen. Finally, ultracentrifugal flotation of plasma from the rabbit injected with I-123-TyC-MBP:hLDL revealed an even division of radioactivity between hLDL and denser plasma fractions.

These in vivo observations suggested either that hLDL particles containing I-123-TyC-MBP are taken up by the liver more rapidly than directly labeled I-123-TyC-hLDL, or that I-123-TyC-MBP is readily stripped from semisynthetic hLDL. That I-123 appeared in non-LDL fractions upon ultracentrifugation further suggested that covalent attachment of I-123-TyC to MBP reduces the affinity of the beta strands for the hLDL surface, perhaps by perturbing the amphiphilicity of their structure.

As an attempt to clarify these issues, we examined a double isotope form of semisynthetic hLDL prepared such that the secondary structure of the MBP and its affinity for hLDL were preserved. Native hLDL was first radiolabeled with I-123-TyC and repurified by Sephadex G-50 chromatography. To this I-123-TyC-hLDL was adsorbed the tritiated nonapeptide [^3H]NH₂-(val-glu-val-ornithine)₂-val-COOH, whose high affinity for the hLDL surface in vitro had been independently established. The complex I-123-TyC-hLDL:[^3H]MBP was recovered by a second round of gel filtration, and injected intravenously into a rabbit. Early gamma camera images showed a similar distribution of the complex to that of I-123-TyC-hLDL without adsorbed MBP.

At about 19 hours postinjection, the rabbit was euthanized. At necropsy organ weights and activities were determined, plasma was subjected to ultracentrifugal flotation, and a portion of liver was homogenized for double isotope counting. Only about 4% of the initially prepared I-123-TyC-hLDL particles adsorbed a molecule of [^3H]MBP; nevertheless, the ratio of tritium:iodine in liver was about 50% higher than in the injected complex, and hLDL remaining in the plasma was profoundly deficient in tritium relative to the injected complex. These findings argue that radioactivity introduced in the form of hLDL-associated MBPs is more rapidly taken up by the liver than is radioactivity introduced as "native" hLDL, regardless of whether the secondary structure of the MBP is perturbed. It was not clear from these data whether MBPs are stripped from circulating hLDL particles, or whether semisynthetic hLDL particles are taken up in toto more rapidly than native hLDL.

B. Radiolabeling and adsorption of betaY.

The experiments described above revealed three technical problems: low labeling efficiency of MBP with I-123-TyC, likely perturbation of MBP secondary structure by I-123-TyC, and low adsorption efficiency of even endogenously labeled MBP to the hLDL surface. We therefore synthesized a new amphiphilic undecapeptide, NH₂-tyr-(ser-val-lys-val)₂-ser-val-COOH, designated betaY, whose aminoterminal tyrosine affords a route for direct radioiodination without perturbing the peptide's secondary structure. A series of experiments with I-125 led to optimized conditions for radioiodination of betaY, adsorption of betaY to hLDL, and recovery of the betaY:hLDL semisynthetic particle. The successful protocol, also used to label betaY with I-131 and with I-123, is given briefly as follows.

Sufficient betaY was labeled to achieve a 1:1 molar ratio of betaY:hLDL in the adsorption mixture. 10-20 nanomoles of betaY (stored as a 0.8 mM stock in 1 mM HCl) was adjusted to pH 7.0 with 20 mM NaOH, and to a final buffer of 2 mM sodium phosphate by addition of 20 mM sodium phosphate, pH 7.0. This solution (about 25 μl total volume) was transferred to an Iodogen-coated Reacti-Vial (Pierce Chemical Co.). After the desired activity of I-125 (as

NaI) was added, the vial was vortexed and let stand at room temperature for 30-60 minutes. The reaction mixture was transferred to a plastic 1.5 ml centrifuge tube, and the labeled peptide was salted away from unreacted radioiodine by adding 200 μ l of 1.0 M NH_4HCO_3 . The pellet was recovered by centrifugation, washed once with 1.0 M NH_4HCO_3 , once with 70% ethanol, and once with diethyl ether. The washed pellet was dried under vacuum, and resuspended in 2 mM HCl. LDL was equilibrated by gel filtration or by ultrafiltration/redilution against 2-5 mM sodium acetate, pH 4.0-5.0. The resuspended radiolabeled betaY was added to the hLDL (about 1 ml final volume), gently mixed, and incubated at room temperature for up to 24 hours. Unbound betaY was removed from betaY:hLDL by gel filtration on Sephadex G-50 equilibrated in physiological phosphate-buffered saline.

Under these conditions, 32-36% of I-125 was bound to betaY, and adsorption of I-125-betaY to hLDL reached a maximum of about 40% within one to two hours. In contrast, we observed less than 10% adsorption under either neutral or basic conditions of incubation, despite using up to 10:1 molar excesses of either hLDL or I-125-betaY.

C. Biodistribution in rabbits of radiolabeled betaY.

For biodistribution analyses betaY was labeled with I-123 or I-131 essentially as described for I-125. Radiolabeled betaY was then either adsorbed to hLDL to generate semisynthetic particles as described, or mixed with 1 ml of nonfasting human serum (as carrier) just prior to injection. Rabbits were injected intravenously with the respective preparations. Blood samples and scintigrams were obtained at selected intervals, and tissues were counted at necropsy between 19 and 48 hours postinjection. The data obtained from four animal studies are summarized and compared to parallel biodistribution results with radiiodine-TyC-hLDL in Tables 1 and 2 and in Figure 1.

For the data presented in Table 1, a chow-fed rabbit was injected intravenously with 105 μ Ci of semisynthetic I-123-betaY:hLDL, and necropsied 19 hours postinjection. Compared to the mean values for three chow-fed rabbits injected with native I-123-TyC-hLDL and necropsied at a comparable time postinjection, the animal receiving semisynthetic hLDL had lower hepatic, intestinal, renal, and gonadal activity, and strikingly lower adrenal and terminal blood activity of I-123.

In Table 2 are displayed tissue and excreted activities for I-131 injected as native I-131-TyC-hLDL, semisynthetic I-131-betaY:hLDL, or uncomplexed I-131-betaY in the presence of carrier human serum. The uncomplexed betaY was injected into both a chow-fed rabbit (Chow) and a rabbit made hypercholesterolemic and atherosclerotic by feeding 1% cholesterol and 4% corn oil for one month (HChol). All animals were necropsied between 42 and 48 hours postinjection. Regardless of the injected form of I-131-betaY, I-131 activities in animals receiving it (compared to the animal receiving native hLDL) were lower for intestine, stomach, kidneys, and gonads; much lower for adrenals and for the terminal blood samples; and uniformly higher for mixed excreta. Hepatic activity was higher in the rabbit receiving semisynthetic hLDL than in either rabbit receiving uncomplexed betaY, but the hypercholesterolemic state may have paradoxically accelerated excretion of

I-131-betaY catabolites. It is also noteworthy, in light of a recent report by Shih et al. (I.-L. Shih, R. S. Lees, M. Y. Chang, and A. M. Lees, Focal accumulation of an apolipoprotein B-based synthetic oligopeptide in the healing rabbit arterial wall, Proc. Natl. Acad. Sci. USA 87:1436 [1990]), that we could not detect by autoradiography any focal uptake of I-131-betaY in aortic atheromata of the hypercholesterolemic rabbit, or in lesion-prone regions of normal aorta.

In Figure 1 are shown blood retention curves of I-123 and I-131 activity for these animals, illustrating the rapid clearance of activity regardless of the form of radioiodine-betaY injected.

Therefore, contrary to our original hypothesis, we conclude that neither semisynthetic betaY:hLDL particles nor betaY alone will likely serve as a useful tracer for hLDL metabolism in vivo.

In terms of potential imaging agents for vascular lesions, in separate experiments we have demonstrated progressive atheroma uptake of In-111-LDL in rabbits with increasing time of exposure, up to 96 hours postinjection, to circulating tracer. From our findings we infer that radiolabeled betaY or semisynthetic hLDL particles are unlikely to survive in the circulation long enough to result in atheroma uptake sufficient for detection by external imaging.

Nevertheless, it is tempting to postulate a mechanism responsible for the unexpectedly rapid metabolism of betaY and other beta-stranded MBPs. Urry (D. W. Urry, Elastomeric polypeptides as potential vascular materials, In Cardiovascular Science and Technology: Basic and Applied I [JC Norman, ed.; Oxymoron Press, Louisville, KY], p. 142) recently reported that naturally occurring beta-stranded sequences of elastin may be chemotactic for vascular endothelial cells. Based on his observation and ours, we propose that betaY, other MBPs, and perhaps even the naturally occurring apoB fragment used by Shih et al.--whether adsorbed to LDL prior to injection or not--may be rapidly swept from the circulation by endothelial cells. Such a phenomenon would account for our findings with I-123-TyC-MBP:hLDL and double-labeled [³H]MBP:I-123-TyC-hLDL; our more recent findings presented here; and both the observation by Shih et al of labeled apoB fragment uptake at the healing edges of ballooned rabbit aortas, and our failure to detect focal uptake by growing rabbit atheromata in which the endothelium is intact.

III. PUBLICATIONS

Manuscripts:

Hay RV, Fleming RM, Ryan JW, Williams KA, Stark VJ, Lathrop KA, Harper PV. 1991. Nuclear imaging analysis of human low density lipoprotein biodistribution in rabbits and monkeys. J Nucl Med, in press.

Abstracts:

Hay RV, Ryan JW, Williams KA, Atcher RW, Brechbiel MW, Gansow OA, Stark VJ, Fleming RM, Donati RJ, Lathrop KA, Harper PV. 1989. Indium-111-[SCN-Bz-DTPA]-low density lipoprotein: A new agent for extended nuclear imaging and

detection of atheromata. In Cardiovascular Science and Technology: Basic and Applied I (JC Norman, ed.; Oxymoron Press, Louisville, KY), pp. 203-204.

Hay RV, Casalino DD, Bassiouny H, Ryan JW, Williams KA, Atcher RW, Brechbiel MW, Gansow OA, Lathrop KA, Fleming RM, Stark VJ, Harper PV. 1990. In vivo accumulation of indium-111-[SCN-Bz-DTPA]-low density lipoprotein by experimental aortic lesions. In Cardiovascular Science and Technology: Basic and Applied II (JC Norman, ed.; Oxymoron Press, Louisville, KY), pp. 73-74.

Hay RV, Ryan JW, Williams KA, Atcher RW, Brechbiel M, Gansow OA, Fleming RM, Stark VJ, Lathrop KA, Harper PV. Submitted. Radiation absorbed dose estimates for radiolabeled human low density lipoprotein.

Book chapters:

Hay R. 1991. The use of radiolabeled low density lipoprotein for evaluating atherosclerosis: present status and future promise, in Progress, Problems and Promise for an Effective Quantitative Evaluation of Atherosclerosis in Animals and Man (RW Wissler, MG Bond, M Mercuri, P Tanganelli, eds; Plenum), in press.

IV. FIGURES

Figure 1: Blood activity retention curves for radioiodine.

Data are graphed as percent injected activity per gram blood, corrected for physical decay, versus time postinjection for rabbits receiving radioiodinated betaY, LDL, or semisynthetic betaY:LDL particles. Solid circles, I-131-TyC-hLDL; open circles, semisynthetic I-131-betaY:LDL; open squares, semisynthetic I-123-betaY:LDL; open triangles, uncomplexed I-131-betaY in chow-fed rabbit; solid triangles, uncomplexed I-131-betaY in cholesterol-fed rabbit.

V. TABLES

TABLE 1.

Biodistribution in Normal Rabbits for I-123 Injected as
Native I-123-TyC-hLDL or as Semisynthetic I-123-betaY:hLDL

Organ	Native	Semisynthetic
Lungs	1.9	0.87
Heart	0.25	0.16
Liver	34	16
Gallbladder	0.75	0.33
Spleen	0.53	0.52
Stomach‡	2.5	4.6
Pancreas†	0.01	0.02
SI‡	2.0	1.4
ULI/cecum‡	12	4.0
LLI‡	0.93	0.87
Kidneys	2.5	0.98
Adrenals	0.87	0.005
Gonads	0.20	0.06
Thyroid	--.--	2.6
Bladder‡	9.4	0.39
Mixed excreta	8.3	20
Blood§	0.16	0.03
Carcass	25	30

Tabulated values express percent administered activity corrected for physical decay (%IA) recovered in the indicated organ at 18-24 hours postinjection, as determined by counting excised organs at necropsy. Values represent the mean for three rabbits injected with native I-123-TyC-hLDL, and the single values at 19 hours postinjection for one rabbit given semisynthetic I-123-betaY:hLDL. †n= 1 for each pancreas value.

‡including contents; §activity per gram blood; SI = small intestine; ULI = upper large intestine; LLI = lower large intestine.

TABLE 2.

Biodistribution in Normal Rabbits for I-131 Injected as
Native I-131-TyC-hLDL, Semisynthetic I-131-betaY:hLDL,
or Uncomplexed I-131-betaY

Organ	Native	Semi-Synthetic	Uncomplexed (Chow)	Uncomplexed (HChol)
Lungs	0.18	2.4	1.6	2.4
Heart	0.06	0.18	0.11	0.06
Liver	19	21	7.8	2.1
Gallbladder	0.02	0.02	0.01	0.005
Spleen	1.1	0.57	0.39	1.0
Stomach‡	2.6	1.4	1.2	0.63
Pancreas	0.008	0.006	0.008	-
SI‡	1.1	0.72	0.44	0.26
ULI/cecum‡	9.7	1.5	1.2	0.63
LLI‡	1.8	0.20	0.20	0.20
Kidneys	1.5	0.37	0.31	0.16
Adrenals	0.39	0.003	0.001	0.004
Gonads	0.09	0.04	0.03	0.009
Thyroid	0.10	5.2	7.2	3.8
Bladder	0.70	0.06	0.17	0.01
Mixed excreta	33	49	63	72
Blood§	0.06	0.005	0.008	0.002
Carcass	14	13	10	5.6

Tabulated values express percent administered activity corrected for physical decay (%IA) recovered in the indicated organ at 42-48 hours postinjection, as determined by counting excised organs at necropsy.

‡including contents; §activity per gram blood; SI = small intestine; ULI = upper large intestine; LLI = lower large intestine.

Radiation Absorbed Dose Estimates for
Radiolabeled Human Low Density Lipoprotein

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We propose a model to generate radiation absorbed dose estimates for radiolabeled low density lipoprotein (*LDL), based upon eight studies of *LDL biodistribution in three adult human subjects. Autologous plasma LDL isolated by ultracentrifugation was labeled with Tc-99m pertechnetate, I-123-tyramine cellobiose, or In-111-isothiocyanatobenzyl-DTPA, and injected intravenously. Biodistribution of each *LDL derivative was monitored by quantitative analysis of scintigrams and direct counting of excreta and of serial blood samples. For each *LDL biological retention of blood activity followed a biexponential curve. By one month postinjection, excretion of *LDL catabolites in urine and stool accounted for up to 92% of injected activity corrected for physical decay (= %IA). Peak hepatic activity was high for each *LDL (5-10 %IA). Tc-99m activity accumulated in kidneys (6-14 %IA), but renal activities for I-123 and In-111 were ≤ 2 %IA. The proposed biodistribution model assumes that transhepatic flux accounts for the majority of *LDL clearance from the bloodstream, and that all other tissue-associated accumulated activity is subject only to physical decay. Stool activity probably reflects biliary excretion of hepatic catabolites of *LDL; however, urine activity may represent intrahepatic conversion of some *LDL catabolites (e.g. via conjugation) to water-soluble form. Values of cumulated activity ($\bar{A}$) and of mean dose per unit administered activity (D) were obtained for each study by application of MIRD conventions and formulae to the model. In each case highest D values were calculated for liver, with mean doses of 5 rads estimated at injected activities of 27 mCi, 9 mCi, and 0.9 mCi for Tc-99m-LDL, I-123-LDL, and In-111-LDL, respectively.

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