The metabolism in vivo and in vitro of plasma low-density lipoprotein from a subject with inherited hypercholesterolaemia

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Summary

1. The metabolism in vivo and in vitro of an abnormal low-density lipoprotein (LDL) obtained from a patient with an inherited form of hypercholesterolaemia was compared with that of LDL obtained from a normal subject.

2. The rates of turnover of the apoprotein of the two types of LDL in a normal subject, and their uptake and catabolism by normal lymphocytes in vitro, were similar.

3. It is concluded that the abnormal behaviour of the patient's LDL may not be due to an abnormality in the apoprotein component.

Key words: lymphocytes, turnover of abnormal low-density lipoprotein.

Introduction

When cultured human fibroblasts are incubated with LDL, the major cholesterol carrier in human plasma, synthesis of HMG-CoA reductase in the fibroblasts is repressed and LDL protein (apoLDL) is catabolized. When cultured fibroblasts from patients with homozygous familial hypercholesterolaemia are incubated with LDL, HMG-CoA reductase remains derepressed and apoLDL catabolism is diminished. From these and other observations Brown, Brannan, Bohmfalk, Brunschede, Dana, Helgeson & Goldstein (1975) have deduced the presence, in human fibroblasts, of genetically controlled surface receptors with high and specific affinity for apoLDL. They postulate that, in fibroblasts, receptor-mediated binding and endocytosis of LDL are essential for the control of intracellular HMG-CoA reductase activity and for catabolism of apoLDL at the normal rate.

Higgins, Lecamwasam & Galton (1975) have reported the presence of an abnormal LDL in two members of a family with primary hypercholesterolaemia. Whereas normal LDL decreased the activity of HMG-CoA reductase in leucocytes from normal subjects and from the index patient, LDL from the index patient had little influence on this enzyme in leucocytes from either source. In terms of the LDL-receptor hypothesis these observations could be explained by supposing that the abnormality in the patients' LDL is such that its protein cannot be recognized by normal LDL receptors. If this is so, the abnormal LDL should not only fail to suppress HMG-CoA reductase activity in cells possessing LDL receptors. Its apoprotein should also be catabolized abnormally slowly in vivo and in vitro. To test this possibility we have compared the metabolism, in vivo and in vitro, of the LDL from the index patient (a male adult) with that of LDL from a normal male adult. Since bile acid sequestrants have been shown to stimulate LDL catabolism (Levy & Langer, 1972), we also tested the effect of Secholex (DEAE-Sephadex, a bile acid sequestrant) on the metabolism of the two specimens of LDL in vivo.

Methods

LDL (d 1.019-1.063), prepared from each subject by
ultracentrifugation (Havel, Eder & Bragdon, 1955), was labelled with $^{125}$I (patient's LDL) or $^{131}$I (normal subject's LDL) (McFarlane, 1964) and prepared for intravenous injection as previously described (Thompson & Myant, 1976). Portions of the two samples of LDL (each containing 2–3 mg of protein with 30 µCi) were mixed and injected intravenously into the normal subject (N.B.M.). Daily blood samples were taken for measurement of the plasma concentrations of $^{125}$I and $^{131}$I. More than 95% of plasma radioactivity was bound to apoLDL, as judged by dextran sulphate/calcium chloride precipitation of the $\rho > 1.006$ plasma fraction (Thompson & Myant, 1976). Total urine was collected in 24 h periods for radioassay. Faeces were collected daily, homogenized with water and a portion of the homogenate was taken for radioassay. During Secholex treatment the daily excretion of $^{125}$I and $^{131}$I in the faeces was 2–4% of that in the urine. Before Secholex treatment, faecal excretion of radioiodide was negligible. The fractional catabolic rates of the two specimens of LDL were calculated from the urine/plasma radioactivity ratios (Thompson & Myant, 1976), corrected for the small amounts of radioactivity excreted in the faeces during the Secholex period. The normal subject took KI (1.2 nmol/day) throughout the study and Secholex (30 g/day) from day 10 to day 18. Antibodies to Australia antigen were not detectable in the serum of the patient.

Lymphocytes were prepared from 300 ml of fresh heparinized normal human blood (Böyum, 1968) and were washed four times in Eagle's minimum essential medium. The cells ($2 \times 10^6$/incubation mixture) were incubated for 2 h at 37°C in the Eagle's medium containing 1% bovine serum albumin and either $^{125}$I-labelled or $^{131}$I-labelled LDL at various concentrations. Surface-bound and total non-lipid radioactivity in the washed cells, and radioactive breakdown products of apoLDL in the medium, were measured by methods described elsewhere (Reichl, Postiglione & Myant, 1976). Radioactivity was measured with a Wallac Instruments double-channel gamma-ray spectrometer.

Results

The two radioactive specimens of LDL disappeared from the circulation of the normal subject at the same rate throughout the whole study, including the period when Secholex was given (Fig. 1). The radioactivity-time curves for both specimens became log-linear by about day 6, with a half-life of 3.9 days. When Secholex was given, the half-life for both LDL specimens decreased to 3.6 days. In keeping with their identical rates of disappearance from the circulation, the rates of accumulation of $[^{125}]$iodide and $[^{131}]$iodide in the urine were similar (Fig. 1). The fractional catabolic rates of the two specimens of LDL were identical throughout the control period, 0.217 ± 0.003 (SEM)/day for the normal LDL and 0.215 ± 0.005/day for the patient's, and both increased significantly during treatment with Secholex: 0.243 ± 0.004/day for the normal LDL and 0.238 ± 0.006/day for the patient's.

When incubated with normal lymphocytes the two specimens of LDL behaved similarly. At LDL concentrations up to 100 µg of protein/ml, approximately equal amounts of the two LDL specimens were surface-bound and approximately equal total amounts of LDL protein (intraplasmic plus surface-bound) were taken up by the cells (Fig. 2). The results obtained at higher LDL concentrations were variable, but there was no suggestion that the
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Fig. 2. Uptake and surface-binding of radiiodine-labelled LDL from the patient and normal subject during incubation with normal lymphocytes. Total uptake of $\bullet$, the patient's and $O$, normal subject's LDL protein; $\Delta$, amount of patient's and $\Downarrow$, of normal subject's LDL protein bound to the surfaces of the lymphocytes. Note the difference in the two vertical scales. Total cell-associated LDL protein was determined from the radioactivity released from the cells after repeated washing with Eagle's minimum essential medium containing $1\%$ albumin. Surface-bound LDL protein was determined from the radioactivity released from the washed cells by brief incubation with trypsin (Bierman et al., 1974). Other details are given in the Methods section.

The identical rates of disappearance of the two labelled LDL specimens from the circulation and the similar rates of accumulation of $^{125}$I- and $^{131}$I-iodide in the urine indicate that the tissues of the normal subject did not distinguish between his own LDL and the patient's LDL towards leucocytes (which has now been observed in the LDL of a third member of the patient's family: M. J. P. Higgins & D. J. Galton, unpublished observations) is not due to a structural abnormality of the apoprotein.

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References


