Effects of Cholestyramine on Receptor-mediated Plasma Clearance and Tissue Uptake of Human Low Density Lipoproteins in the Rabbit*

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This study examines the effects of cholestyramine (2 g/day) on the plasma clearance and tissue uptake of human low density lipoprotein (LDL) in rabbits. 1,2-Cyclohexanedione modification of human LDL abolishes its recognition by high affinity cell membrane receptors in vitro and degrades its plasma clearance in comparison to native LDL. Consequently, the difference between the fractional rates of catabolism of simultaneously injected native and cyclohexanedione-treated LDL is an index of in vivo receptor-mediated clearance of the lipoprotein. When human 125I-LDL and 131I-cyclohexanedione-treated LDL were injected into rabbits, 44% of the lipoprotein was cleared from the plasma by the receptor mechanism. Various tissues were removed from the animals at the end of the turnover study and their relative uptakes of 125I native and 131I-cyclohexanedione-treated LDL were measured. All exhibited receptor activity to some extent, incorporating more native than cyclohexanedione-modified LDL. The greatest receptor activity per g of tissue was found in lymph nodes, spleen, and liver and, in terms of whole organ uptake, the liver played a major role in LDL catabolism. Treatment of the rabbits with cholestyramine lowered the circulating LDL cholesterol level by promoting its clearance (120%, p < 0.001) via the receptor pathway. This was associated with a virtual doubling of receptor-mediated incorporation of the lipoprotein into the liver. These results suggest that the drain which cholestyramine induces in the hepatic cholesterol pool promotes LDL receptor activity in this organ and thereby lowers the level of circulating LDL.

MAMMALIAN cells in culture can fulfil their requirements for cholesterol by assimilating it from cholesteryl ester-rich low density lipoprotein (LDL) particles in the culture medium (1). This is achieved by the agency of a specific high affinity membrane receptor which recognizes positively charged amino acid residues on the lipoprotein surface (2, 3). Chemical modification of these residues abolishes the receptor-lipoprotein interaction. Persistent residual catabolism is due to non-specific uptake of the lipoprotein by low affinity mechanisms (3).

Extrapolation from these tissue culture studies to the whole organism has led to the postulation of two catabolic routes for LDL in vivo, one via the receptor pathway and the other receptor-independent (4). In an earlier report (5), we attempted to determine the relative contribution of each pathway to the catabolism of LDL using 1,2-cyclohexanedione-modified lipoprotein. Such treatment inhibits LDL degradation via the receptor path while receptor-independent clearance is not apparently affected (3, 6). Our findings indicated that the receptor route accounts for 33% of LDL catabolism in normal individuals and for 16% in heterozygous familial hypercholesterolemic subjects. The latter are commonly treated by administration of the bile acid sequestrant cholestyramine which effectively lowers plasma cholesterol by promoting LDL receptor activity (6). Many tissues, including the liver, contain high affinity receptors for LDL (7) and could be responsible for its enhanced degradation during cholestyramine treatment. However, since the drug is not absorbed into the systemic circulation (8) but acts primarily to deplete the hepatic cholesterol pool, we speculated that the observed increase in receptor activity might be localized in the liver. To test this hypothesis, we have examined the effects of the drug on plasma clearance and tissue uptake of human LDL in rabbits.

MATERIALS AND METHODS

Animals—Four-month-old male New Zealand White rabbits, maintained ad libitum on a commercially available diet (S.G.I., Oxford, Ltd., Basingstoke, Hampshire, U.K.) were used in the study. They were assigned arbitrarily to a control or treated group. The animals in the latter received a daily dose of 2 g of cholestyramine (Bristol Laboratories, Stamford House, Langley, Slough, U.K.) suspended in 10 ml of water by mouth for 21 days before and throughout the turnover study. The hypocholesterolemic action of the drug was determined by measuring plasma lipids and lipoproteins (9) on 14-h fasting blood samples collected from the marginal ear vein on three occasions at weekly intervals during treatment.

Preparation and Chemical Modification of Labeled LDL—Human LDL (1.030 < d < 1.050 kg/liter) was isolated from normal plasma by rate zonal ultracentrifugation (10), dialyzed against 0.15 M NaCl/0.01% Na2EDTA, pH 7.0, and divided into two aliquots, one of which was labeled (11) with 125I and the other with 131I (The Radiochemical Centre, Amersham, U.K.). The labeled lipoproteins were then freed of unbound radioiodide and glycine buffer by exhaustive dialysis against 0.15 M NaCl/0.01% Na2EDTA, pH 7.0, followed by gel filtration through Sephadex PD-10 columns (Pharmacia GB, Ltd., London, U.K.). The 125I-LDL was treated with 1,2-cyclohexanedione (Fluorochem, Ltd., Glossop, U.K.) as described elsewhere (5) to block the charge on the arginyl residues of its protein moiety. Unreacted cyclohexanedione was removed by dialysis of the reaction mixture against 0.15 M NaCl/0.01% Na2EDTA, pH 7.0. Reversal of the isotopes in different studies did not affect the results. The labeled lipoprotein preparations have been fully characterized previously (5).

Turnover Study Protocol—Two days prior to and throughout each turnover study, the rabbits were given 0.1 g/liter of KI in their drinking water to prevent thyroidal sequestration of radioiodide. Approximately 10 μCi (300 μg of protein) each of LDL and cyclohexanedione-treated LDL labeled with different isotopes of iodine were
mixed, sterilized by membrane filtration (0.22 μ filters, Millipore Corp., Bedford, MA) and injected intravenously into the marginal ear vein of each rabbit in the control and treatment groups. Blood samples were then collected from the opposite ear after 10 min and subsequently on eight occasions over the next 48 h. The isotope dilution which occurred in the first 10 min provided an estimate of plasma volume. Plasma decay curves for both isotopes were constructed and used to calculate the fractional clearance rate of each tracer (12).

Tissue Uptake of LDL and Cyclohexanedione-treated LDL.—The tissue distribution of each iodine isotope was measured in the control and test rabbits immediately after collection of the final blood sample (i.e. 48 h post isotope injection). The rabbits first received an intravenous dose of 5000 units of sodium heparin (Weddel Pharmaceuticals Ltd., London, U.K.) to prevent intravascular coagulation, followed 5 min later by 30 mg/kg of pentobarbitone sodium (May and Baker, Ltd., Dagenham, U.K.). Exsanguination was then performed by cardiac puncture which consistently removed more than 80% of the animals’ total blood volume. Individual organs were dissected out, washed in ice-cold 0.15 M NaCl, blotted dry, and weighed. Tissue samples of approximately 1 g wet weight were then taken in quadruplicate and weighed, and their 125I and 131I radioactivity was determined in a twin channel γ spectrometer (Packard Instruments, Downers Grove, IL). The radioactivities were calculated per g wet weight of tissue and were expressed relative to the measured total plasma radioactivity of the respective isotope at the time of sacrifice.

**RESULTS**

Cyclohexanedione modification of human LDL delayed its clearance from the plasma of rabbits (Fig. 1). On the assumption that the removal rate of the modified lipoprotein represents receptor-independent catabolism (6), then the difference between this value and the fractional clearance rate of untreated LDL is a measure of receptor-mediated catabolism. In a series of 14 control rabbits (Table I), receptor-mediated clearance accounted for 44% of the total fractional catabolism of 1.49 ± 0.19 pools/day (mean ± S.D.). Similar mean values for total (1.53 ± 0.23 pools/day) and receptor-independent

![Graph](image-url)

Fig. 1. Effects of cholestyramine on plasma decay curves of untreated and cyclohexanedione-modified human LDL in rabbits. Human 125I-LDL and 131I-cyclohexanedione-modified LDL (200 μg of protein) were injected into the plasma of seven control and six cholestyramine-treated (2 g/day) rabbits via a marginal ear vein. Plasma samples were removed from the opposite ear after 10 min and then at frequent intervals over the next 48 h. Each point, with its error bar, represents the mean ± S.D. for the group. CHD, 1,2-cyclohexanedione.

### Table I

| Rabbit | Receptor-independent | Receptor-mediated |
|--------|----------------------|-------------------|
|        | pools/day             | pools/day         |
| 1      | 0.98                 | 0.62              |
| 2      | 1.08                 | 0.56              |
| 3      | 1.00                 | 0.79              |
| 4      | 0.68                 | 0.80              |
| 5      | 0.75                 | 0.58              |
| 6      | 0.77                 | 1.11              |
| 7      | 0.62                 | 0.75              |
| 8      | 0.80                 | 0.73              |
| 9      | 0.82                 | 0.52              |
| 10     | 0.82                 | 0.58              |
| 11     | 0.83                 | 0.40              |
| 12     | 0.68                 | 0.80              |
| 13     | 0.93                 | 0.57              |
| 14     | 0.83                 | 0.42              |

Mean ± S.D. 0.84 ± 0.13 0.66 ± 0.19

* Plasma fractional clearance rate of 1,2-cyclohexanedione-treated LDL.
* Plasma fractional clearance rate of native LDL minus that of 1,2-cyclohexanedione-treated LDL.

### Table II

| Parameter | Weeks |
|-----------|-------|
|           | 0     | 1     | 2     |
| Plasma cholesterol (mg/dl) | 87 ± 20 | 47 ± 9* | 44 ± 11* |
| LDL cholesterol (mg/dl)  | 36 ± 10 | 8 ± 3* | 5 ± 3* |
| High density lipoprotein cholesterol (mg/dl) | 34 ± 9 | 32 ± 7 | 36 ± 7 |

* Paired t test versus Week 0, p < 0.001.

### Table III

| Rabbit | Fractional clearance rate of LDL |
|--------|---------------------------------|
|        | Receptor-independent | Receptor-mediated |
|        | pools/day             | pools/day         |
| 15     | 0.96                 | 1.62              |
| 16     | 0.90                 | 1.54              |
| 17     | 0.71                 | 1.14              |
| 18     | 0.84                 | 1.35              |
| 19     | 0.89                 | 1.18              |
| 20     | 1.09                 | 1.87              |

Mean ± S.D. 0.90 ± 0.13 1.40 ± 0.28

* Plasma fractional clearance rate of 1,2-cyclohexanedione-treated LDL.
* Plasma fractional clearance rate of native LDL minus that of 1,2-cyclohexanedione-treated LDL.
* Unpaired t test versus controls (Table I), not significant.
* Unpaired t test versus controls (Table I), p < 0.001.

Cholestyramine therapy (2 g/day) lowered plasma cholesterol in the rabbits by 50% by reducing the level of circulating LDL cholesterol (Table II). High density lipoprotein cholesterol remained unchanged. Consequently, the rabbit responds
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to the drug similarly to humans (13). Likewise, as in humans, treatment increased the fractional removal rate of LDL from the plasma by promoting specifically its receptor-mediated clearance (6). This rose from a control value of 0.86 ± 0.19 pools/day (n = 14) to 1.45 ± 0.28 pools/day (n = 6), while receptor-independent catabolism, measured by the clearance of modified LDL, was unaffected by the drug (Fig. 1; Tables I and III).

Table IV describes the relative uptake of LDL via receptor and nonreceptor pathways into various rabbit tissues. The values were calculated as follows. First, in order to permit comparison of uptake of the two isotopes, $^{125}$I or $^{131}$I radioactivities per g wet weight of tissue were expressed relative to the total plasma $^{125}$I or $^{131}$I radioactivity measured at the same time. The value obtained for the isotope bound to cyclohexanedione-treated LDL was taken to represent "receptor-independent" uptake of the lipoprotein (Table IV). This includes nonspecific incorporation into cells plus contamination from plasma and interstitial fluid. The difference between this value and that calculated for native LDL ("receptor-mediated" uptake, Table IV) was taken to be a measure of receptor activity in each tissue.

In all tissues examined, the uptake of native LDL exceeded that of the cyclohexanedione-treated lipoprotein, that is all demonstrated receptor activity to some extent. Lymph nodes, spleen, and liver were most active in this regard and fat, muscle, and thymus were least active.

Cholestyramine treatment promoted hepatic uptake (86%, p < 0.05) by the receptor mechanism without affecting receptor-independent incorporation. No other tissues demonstrated a significant change in uptake by either route during drug treatment.

When receptor-mediated incorporation into discrete organs of both control and cholestyramine-treated animals was expressed (Table V) in terms of the whole organ (i.e. the uptake per g wet weight x the total organ weight), the liver emerged as the most active organ of this group. Moreover, uptake into the liver by the receptor route was positively correlated with receptor-mediated LDL clearance from the plasma (r = 0.86, p < 0.001, Fig. 2). No other organ showed a similar relationship to plasma clearance.

**DISCUSSION**

Cholestyramine is a widely used hypcholesterolemic agent which functions by binding bile acids in the gut and interrupting their enterohepatic circulation (14). This action has been shown in animals to produce an increase in the activity of 7a-hydroxylase, the rate-limiting enzyme in bile acid synthesis, leading to a depletion in the hepatic cholesterol pool (15). A similar effect on bile acid production has been observed in man (16). The hypercholesterolemic action of the drug is based on its influence on LDL metabolism. Specifically, it increases the fractional and absolute rates (6) of LDL catabolism via the high affinity receptor pathway described by Goldstein and Brown (1, 4). Since this pathway appears to be widely distributed among the various tissues of the body (1, 4).
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7), the question remains whether the response to cholestyramine is equally disseminated throughout these tissues or localized to a particular site. Tissue culture studies of the LDL receptor pathway (1, 4) indicate that cells will respond to cholesterol depletion by increasing their assimilation of LDL (7), the drain on the hepatic cholesterol pool caused by the drug should promote receptor-mediated uptake of the lipoprotein into this organ.

This kind of mechanism seems to operate in male rats exposed to high doses of estradiol. The hormone induces a pronounced hypolipoproteinemia. In particular, the LDL level is reduced due to an increase in its plasma clearance rate and hepatic uptake (17), apparently via promotion of receptor activity (18). Receptor-independent clearance of plasma LDL, as measured by the removal rate of the cyclohexanedione-modified lipoprotein, remains unaffected by treatment (18).

The present study examines the influence of cholestyramine on receptor-mediated LDL catabolism and uptake into various tissues of the rabbit. The response of this animal to drug treatment was very similar to that of humans both in terms of reduction in lipoprotein cholesterol levels and an increase in receptor-mediated LDL catabolism (Fig. 1; Tables I, II, and III). Furthermore, initial studies indicated that there was no difference in the metabolic handling of rabbit and human LDL in this animal.

In order to establish the role of each tissue in LDL catabolism, we measured the plasma clearance and tissue uptake of native and cyclohexanedione-treated LDL, basing our rationale on the findings of Mahley et al. (2) that this chemical modification of LDL inhibits its receptor recognition and prevents high affinity binding and uptake into cells. We argued that the amount of radioactivity associated with cyclohexanedione-treated LDL in a tissue specimen should account for nonspecific incorporation of the lipoprotein (including that in trapped plasma and interstitial fluid, and any labeled tracer incorporated into the cells by a low affinity, nonreceptor route). Any increase in the uptake of native LDL over this value should reflect receptor activity and consist of label in the liver compartments (12), it was possible to compare receptor activity from organ to organ, assuming that each cleared isodotyrosine at a similar rate.

In all tissues examined, uptake of native LDL exceeded that of cyclohexanedione-modified LDL, suggesting that receptor activity is widespread throughout the tissues of the body, in confirmation of an earlier report (7). Certain tissue, specifically lymph nodes, spleen, liver, and kidney are particularly active in this regard (Table IV), as was found by Pittman and coworkers (19) in a study of total LDL degradation by various tissues in the pig. When receptor-mediated LDL uptake is expressed in terms of the whole organ, the liver emerges as a major contributor to the catabolism of the lipoprotein (Table V). In fact, hepatic receptor activity is strongly positively correlated with receptor-mediated plasma clearance (Fig. 2), adding support to the observations of others (19–21) that the liver is an important determinant of LDL clearance from the plasma pool.

It also appears that this organ is central to the hypocholesterolemic action of cholestyramine. The specific drug-induced increase in receptor-mediated LDL clearance from the plasma (Fig. 1) was associated with a virtual doubling of hepatic LDL uptake by the receptor route (Table IV) without significant change in any of the other tissues examined. In theory, this could result from a primary increase in hepatic receptor activity or from altered lipoprotein-receptor affinity secondary to the known increment in the LDL protein/cholesterol ratio which is induced by cholestyramine treatment (6). We favor the former possibility since it has been shown (22) that a compositional change in LDL of similar magnitude to that produced by cholestyramine therapy does not affect its receptor binding affinity. In light of this, we believe that our observations support the hypothesis that the drug, by its action on cholesterol metabolism in the liver, stimulates hepatic receptor activity and thereby causes directly a reduction in the circulating LDL level.

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