In vivo catabolism of human low density lipoprotein in the rat is mediated by a nonsaturable, low-affinity mechanism

F.M. van 't Hooft and A. van Tol*

Department of Biochemistry I, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands

Received 23 January 1985; revised version received 18 February 1985

The degradation of human low density lipoprotein (LDL) was analyzed in fasted rats treated for 3 days with either 4-aminopyrazolo-(3,4-d)pyrimidine (4APP) or saline. Treatment with 4APP caused an 80% decrease in serum cholesterol concentration. The mono-exponential serum decay of a tracer amount of labelled LDL was changed neither by 4APP treatment, nor by the simultaneous injection of a bolus of unlabelled LDL. The sites of degradation of human LDL were determined using the nondegradable labelling compound *O*-(4-diazo-3-[^{125}I]iodobenzoyl)sucrose (D^{125}IBS). The sites of degradation and the rate of degradation of D^{125}IBS labelled LDL were also not affected by 4APP treatment or by injection of a bolus of unlabelled LDL. It is concluded that human LDL is catabolised in the rat by way of a nonsaturable, low-affinity mechanism.

Low density lipoprotein *4-Aminopyrazolo-(3,4-d)pyrimidine* *Catabolic site* *LDL receptor* *B/E receptor* *O-(4-Diazo-3-[^{125}I]iodobenzoyl)sucrose*

1. INTRODUCTION

The catabolic pathway of serum LDL has been studied extensively by the group of Brown and Goldstein (review [1,2]). It was shown that LDL catabolism is initiated by the binding of LDL to a saturable, high-affinity receptor (LDL- or B/E-receptor) on the cell surface. There is considerable evidence that the number of LDL receptors is regulated by the cellular need for cholesterol. In the absence of LDL receptors (homozygous familial hypercholesterolemia) LDL is degraded by a nonsaturable, low-affinity mechanism.

The rat has been used extensively as an experimental model for the study of lipoprotein metabolism in vivo. Because the plasma LDL concentration in the rat is very low, human LDL was used instead in most of these metabolic studies. However, several in vitro binding studies have provided evidence that human and rat LDL bind with different binding characteristics to LDL receptors in rat tissues [3,4]. Moreover, it has repeatedly been observed that the serum decays of human and rat LDL in the rat are not identical [5,6]. These studies indicate that human and rat LDL are not metabolised by the same catabolic pathway in the rat. Here, we evaluated the mechanisms of human LDL catabolism in the rat.

2. MATERIALS AND METHODS

Male retired breeders of the Wistar strain, weighing 300–400 g, were used in all experiments. One ml of 4APP (Aldrich-Europe, Beerse, Belgium), dissolved in saline, was administered intraperitoneally without anaesthesia at a concentration of 20 mg/kg for 3 consecutive days. Control animals were injected with saline. All animals were given free access to water, but food was withheld. Body weights of the rats treated with 4APP

* To whom correspondence should be addressed

Abbreviations: LDL, low density lipoprotein; DIBS, *O*-(4-diazo-3-iodobenzoyl)sucrose; 4APP, 4-aminopyrazolo-(3,4-d)pyrimidine

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30 © 1985 Federation of European Biochemical Societies

decreased from 356 ± 22 g to 316 ± 19 g (n = 27) and of control rats from 347 ± 23 g to 312 ± 23 g (n = 9).

Human LDL, isolated by sequential ultracentrifugation, was iodinated with ^{131}I using the ICl method and with D^{125}IBS, as described [7]. The radiolabelled LDLs were mixed and 'screened' in vivo, as described [7], except that it was performed in rats treated with 4APP. One min prior to the intravenous injection of labelled LDL under light ether anaesthesia, 2 ml of either unlabelled LDL (containing about 12.5 mg LDL cholesterol) or Krebs-Henseleit buffer [8] were injected into a femoral vein. The mixture of ^{131}I-LDL and D^{125}IBS-LDL was injected into the contralateral vein in a volume of 1 ml. Three min after the injection of labelled LDL a blood sample was obtained from the orbital plexus behind the eye. The radioactivity in this sample was taken to be the initial serum radioactivity for the calculation of the serum decay. 0.25, 1 and 4 h after injection of labelled LDL the animals were anaesthetised with ether and bled from the abdominal aorta. The blood was allowed to clot at room temperature and serum was isolated by low speed centrifugation. A number of tissues (heart, lungs, liver, spleen, kidneys, jejunum, adipose tissue, muscle, hide, adrenals and testes) were excised, weighed and counted for radioactivity. The DIBS-dependent accumulation in each tissue was calculated as in [7]. Na^{125}I (350–600 mCi/ml) and Na^{131}I (40 mCi/ml), both carrier-free, were obtained from Amersham International, Amersham, England. Cholesterol was measured by an enzymatic method as described [9]. All data presented are means ± SD with the number of experiments in parentheses.

3. RESULTS AND DISCUSSION

When iodinated human LDL was injected intravenously in tracer amounts in the rats used in this study, a monoexponential serum decay was observed during the 4 h experiment with a $t_{1/2}$ of 9.4 ± 0.2 h (n = 3), measured by extrapolation until 50% of the injected dose was removed from the serum compartment. This value is in excellent agreement with data obtained from long-term experiments in male rats fasted overnight. In these animals we also measured monoexponential serum decays of human ^{125}I-LDL ($t_{1/2}$ 10.0 ± 0.7 h; n = 3). The serum radioactivity was measured at 1, 4, 8, 20 and 28 h after injection of ^{125}I-LDL. When a bolus injection of unlabelled human LDL was given intravenously, 1 min prior to the injection of labelled LDL, the serum cholesterol concentration was raised approx. 3-fold and remained elevated for at least 4 h (fig.1). However, the serum decay of labelled human LDL was essentially unchanged with a $t_{1/2}$ of 9.7 ± 0.4 h (n = 3). This indicates that the catabolic pathway of human LDL in the rat is not saturable, not even at this, for the rat, extremely high serum LDL concentration.

Kovanen et al. [10] showed that the LDL receptor for human LDL in liver membranes isolated from normal rats is virtually nondetectable. Therefore we thought of ways to increase the LDL receptor level and LDL degradation in the rat. Cells in tissue culture are able to increase the number of LDL receptors, and therefore the capacity to degrade LDL, in response to incubation in lipoprotein-deficient medium [1,2]. In vivo experiments showed that 4APP dramatically reduces the serum cholesterol concentration in the rat [11,12], probably by inhibition of lipoprotein synthesis and secretion from the liver. It was also shown that 4APP treatment increases LDL binding in the adrenal glands of the mouse [13]. These studies suggested that 4APP treatment could increase the rate of catabolism of LDL in vivo. However, in this study it was found that the serum decay of labelled human LDL from rat serum was not influenced at all by 4APP treatment. The

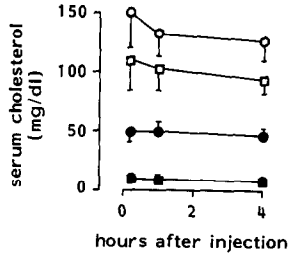

Fig.1. Serum cholesterol concentrations. The serum cholesterol concentrations of control rats injected buffer (●) or unlabelled human LDL (○), and of 4APP-treated rats injected buffer (■) or unlabelled human LDL (□) were measured by an enzymatic method [9]. Results are means ± SD for 3 experiments.

serum decay of iodinated human LDL in 4APP-treated animals had a $t_{1/2}$ of 9.3 ± 0.2 h (n = 3), indicating that the catabolic pathway for human LDL in the rat cannot be upregulated in vivo by 4APP treatment. When unlabelled LDL was injected into 4APP treated rats just prior to the injection of iodinated LDL, the plasma cholesterol concentration was raised to 100 mg/dl (fig.1). However, the serum decay of labelled human LDL was not affected and the $t_{1/2}$ was 9.5 ± 0.3 h (n = 3), indicating again that the catabolic pathway of human LDL in the rat is not saturable. Also the disappearance rate of injected unlabelled human LDL-cholesterol is essentially unchanged by 4APP treatment (see fig.1).

The sites of catabolism of the protein moiety of human LDL were analysed with the nondegradable labelling compound DIBS. In agreement with [7], we observed no difference between the serum decay of conventionally iodinated LDL and DIBS-labelled LDL (not shown), indicating that the DIBS label does not affect the in vivo turnover of LDL. Recently we observed that DIBS-labelled human LDL is catabolised predominantly in the rat liver [7], as shown previously with sucrose-labelled human LDL [14,17]. Here, it was found that under all conditions (control rats, control rats + unlabelled human LDL, 4APP-treated rats, 4APP-treated rats + unlabelled LDL) the liver remained the most prominent site of catabolism of human LDL. The DIBS-dependent accumulation of radioactivity, an index of the rate of catabolism of LDL, was calculated for each tissue as described in [7]. There were no statistically significant differences in the DIBS-dependent accumulation in the liver after injection of radiolabelled LDL under any of the conditions mentioned above (fig.2). In agreement with the data obtained from the serum decays of radioactive human LDL, there is therefore no evidence that the rate of catabolism of labelled LDL in the liver is influenced by the serum LDL concentration or by 4APP treatment.

Besides the liver the only tissue that showed any DIBS-dependent accumulation of radioactivity was the spleen. 4 h after injection of labelled LDL this tissue only accumulated less than 0.5% of the injected dose, and this percentage was not changed by a bolus injection of unlabelled LDL or 4APP treatment.

Evidence suggesting that the adrenal gland is,

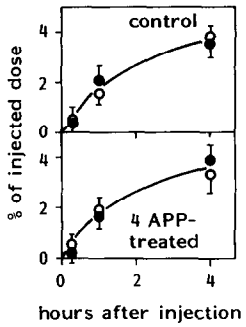

Fig.2. DIBS-dependent accumulation of LDL-derived radioactivity in the liver. The DIBS-dependent accumulation of LDL radioactivity in the liver of control rats injected buffer (●) or unlabelled human LDL (○), and of 4APP-treated rats injected buffer (●) or unlabelled LDL (○) was calculated as described in [7] by subtracting the percentage of the injected dose of the ^{131}I-labelled LDL from that of the injected dose of the D^{125}IBS-labelled LDL present in the liver at the indicated times. Results are expressed as means ± SD for 3 experiments.

per g tissue, by far the most active organ for LDL binding has been presented [15]. However, in agreement with [7], we observed no significant DIBS-dependent accumulation of LDL-derived radioactivity during the 4 h experiment in the adrenal glands of control rats and of control rats injected with a bolus of unlabelled LDL. 4APP treatment causes an almost 2-fold increase in the wet weight of the adrenal glands (not shown). It is not clear why the adrenal gland increases in size in response to 4APP treatment, but it can be speculated that it is a compensatory reaction of the tissue to the hypocholesterolemia, because the adrenal glands of rats treated with 17α-ethinylestradiol showed a similar increase in wet weight. As shown in fig.3, the LDL-derived radioactivity in the adrenal glands of animals treated with 4APP increases approx. 5-fold, if compared with the radioactivity in the adrenal glands of control animals. The adrenal gland was the only tissue in which this increase was observed. However, the radioactivity associated with the adrenal glands could not be diminished by a bolus injection of unlabelled LDL. The increased association of labelled LDL with the adrenal glands of 4APP-treated animals cannot be fully explained by the increased adrenal weight, but the in-

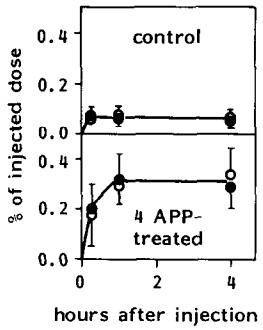

Fig.3. Accumulation of LDL-derived radioactivity in the adrenal glands. Accumulation of intravenously injected D^{125}IBS-labelled LDL in the adrenal glands of rats injected with buffer (●) or unlabelled human LDL (○), or of 4APP treated rats injected buffer (●) or unlabelled LDL (○) is expressed as percent of the injected dose. Values are means ± SD for 3 experiments.

ability to decrease the radioactivity with excess unlabelled LDL indicates that this LDL binding is not saturable. It is unlikely that the increased LDL binding is related to LDL degradation, because DIBS-dependent accumulation could not be detected in the adrenal glands of 4APP-treated animals.

Our data indicate that the catabolic pathway of human LDL in the rat is not saturable by the injection of a high dose of unlabelled LDL, a conclusion reached before by others [16], using a different methodology. Moreover, the present data show that the catabolic pathway of human LDL in the rat cannot be upregulated by a prolonged reduction of the plasma cholesterol concentration caused by 4APP treatment. It is therefore likely that human LDL is not catabolised in the rat by way of a saturable, high-affinity mechanism, but by a nonsaturable, low-affinity process. This conclusion seems to be at variance with the results of earlier studies which showed that methylated human LDL is removed slower from rat serum than native human LDL [6,17,18]. However, using the same chemical modification, Koelz et al. [16] could not find any evidence in favor of a receptor-mediated clearance of human LDL by the rat liver or adrenal glands. In addition Chao et al. [19] reported that the removal of cyclohexanedione-modified ^{125}I-LDL from the blood of control rats is not very different from that of native ^{125}I-LDL.

It is evident that the results obtained with chemically modified human LDL in the rat are not consistent. These experiments are based on the assumption that chemically modified LDL and native LDL interact in the same way with LDL receptors in the rat in vivo as they do with LDL receptors in vitro. It is also assumed that chemically modified human LDL is not recognized as foreign material and therefore rapidly removed from the circulation. In this respect it could be of importance that the $t_{1/2}$ of about 10 h for native human ^{125}I-LDL observed in this study is higher than the $t_{1/2}$ of 1–3 h for the initial rapid phase observed by others [6,14]. In this study a different approach was used to study the mechanism of human LDL catabolism in the rat, which is based on the notion that a receptor-mediated process is characterised by the presence of a competition of a large bolus of unlabelled LDL with a tracer amount of labelled LDL. Our study indicates that the in vivo catabolism of human LDL in the rat is not mediated by the 'classical' LDL receptor pathway as described by Brown and Goldstein [1,2]. It is thought that the information for the LDL receptor protein, although present on the rat genome, hardly results in functioning receptors for human LDL at the liver cell surface [10,20]. The LDL receptor is induced only after treatment of rats with very high doses of 17α-ethinylestradiol. This results in a 3–10-fold increase in the binding of human LDL to liver membranes and a 12-fold increase in the hepatic uptake of intravenously administered human LDL [10]. Human LDL has been used in most experiments dealing with LDL metabolism in the rat. The results of the present study underline the importance of the source of lipoproteins (homologous vs heterologous) in metabolic studies.

ACKNOWLEDGEMENTS

We thank John F.C. Molenaar and Teus van Gent for excellent technical assistance and Martha I. Wieriks for typing the manuscript. These studies were supported by grant 30.002 from the Dutch Heart Foundation.

REFERENCES

[1] Brown, M.S., Kovanen, P.T. and Goldstein, J.L. (1981) Science 212, 628–635.

[2] Brown, M.S. and Goldstein, J.L. (1983) J. Clin. Invest. 72, 743–747.

[3] Innerarity, T.L., Pitas, R.E. and Mahley, R.W. (1980) J. Biol. Chem. 255, 11163–11172.

[4] Drevon, C.A., Attie, A.D., Pangburn, S.H. and Steinberg, D. (1981) J. Lipid Res. 22, 37–46.

[5] Van Tol, A., Van 't Hooft, F.M. and Van Gent, T. (1978) Atherosclerosis 29, 449–457.

[6] Mahley, R.W., Weisgraber, K.H., Melchior, G.W., Innerarity, T.L. and Holcombe, K.S. (1980) Proc. Natl. Acad. Sci. USA 77, 225–229.

[7] Van 't Hooft, F.M. and Van Tol, A. (1985) FEBS Lett. 179, 225–228.

[8] Krebs, H.A. and Henseleit, L. (1932) Hoppe-Seyler's Z. Physiol. Chem. 218, 33–66.

[9] Röschlau, P., Bernt, E. and Gruber, W. (1974) Z. Klin. Chem. Klin. Biochem. 12, 403–409.

[10] Kovanen, P.T., Brown, M.S. and Goldstein, J.L. (1979) J. Biol. Chem. 254, 11367–11373.

[11] Shiff, T.S., Roheim, P.S. and Eder, H.A. (1971) J. Lipid Res. 12, 596–603.

[12] Andersen, J.M. and Dietschy, J.M. (1977) J. Biol. Chem. 252, 3652–3659.

[13] Kovanen, P.T., Goldstein, J.L., Chapell, D.A. and Brown, M.S. (1980) J. Biol. Chem. 255, 5591–5598.

[14] Pittman, R.C., Attie, A.D., Carew, T.E. and Steinberg, D. (1982) Biochim. Biophys. Acta 710, 7–14.

[15] Brown, M.S., Kovanen, P.T. and Goldstein, J.L. (1980) Ann. NY Acad. Sci. 348, 48–68.

[16] Koelz, H.R., Sherrill, B.C., Turley, S.D. and Dietschy, J.M. (1982) J. Biol. Chem. 257, 8061–8072.

[17] Carew, T.E., Pittman, R.C. and Steinberg, D. (1982) J. Biol. Chem. 257, 8001–8008.

[18] Harkes, L. and Van Berkel, Th.J.C. (1984) Biochem. J. 224, 21–27.

[19] Chao, Y., Windler, E.E., Chen, G.C. and Havel, R.J. (1979) J. Biol. Chem. 254, 11360–11366.

[20] Kroon, P.A., Thompson, G.M. and Chao, Y.S. (1984) Biochem. J. 223, 329–335.