Visualization of the hepatic low-density lipoprotein receptor in rats by sequential scintiscans

E. Friedrich, H.A. Dresel,* H. Sinn and G. Schettler*

Deutsches Krebsforschungszentrum, Institut für Nuklearmedizin, Im Neuenheimer Feld 280, D-6900 Heidelberg, and **Medizinische Klinik der Universität Heidelberg, Bergheimer Straße 58, D-6900 Heidelberg, FRG*

Received 2 February 1985; revised version received 18 March 1985

The usefulness of a gamma camera system for external imaging of the degradation sites of low-density lipoprotein (LDL) is demonstrated in rats. [^{131}I]LDL was injected intravenously in normal rats and rats pretreated with 17α-ethinyl estradiol for LDL receptor induction. Distribution of the radioligand was followed for 20 min, for perfusion control [^{131}I]albumin was administered. Rats pretreated with 17α-ethinyl estradiol showed a marked increase in the LDL uptake by the liver, which was not competed by the preinjection of physiological amounts of unlabelled LDL. It is concluded that the injection of small amounts of [^{131}I]LDL is sufficient to image the hepatic LDL receptor and that the endogenous LDL does not compete effectively at physiological LDL levels.

Low-density lipoprotein *LDL receptor* *17α-Ethinyl estradiol* *Scintigraphy*

1. INTRODUCTION

It is well documented in studies done in vivo and in vitro that the liver of rat pretreated with 17α-ethinyl estradiol expresses membrane receptors capable of binding human low-density lipoprotein (LDL) which mediates the uptake and degradation of these particles by the hepatocytes [1–4]. In the estradiol-treated rat the liver is the major catabolic compartment for the degradation of intravenously injected human LDL. In contrast, in normal rats there are only small numbers of hepatic LDL receptors. After intravenous injection of human LDL into normal rats, the catabolism of LDL is directed mainly by receptor-independent mechanisms. As a result, the removal of LDL from the plasma compartment is slow. Thus, 17α-ethinyl estradiol-treated rats and normal rats as a control might provide favorable circumstances to study the kinetics of the LDL receptor mediated uptake of LDL in the rat liver.

The need for external but sensitive methods to study the regulation of the metabolism of lipoproteins in vivo prompted us to investigate the feasibility of scintiscans to evaluate receptor activity in vivo. We labelled human LDL with [^{131}I]iodine to obtain sufficiently high specific activities by a modification of the method of Mc Farlane [5] and injected the radioligands into the rats. As an indicator for organ perfusion, [^{131}I]albumin was used. Here we compare the uptake of [^{131}I]LDL by the liver in normal rats and rats pretreated with 17α-ethinyl estradiol which was continuously followed by a gamma camera interfaced with a computer. The results of this study allow us to suggest that it is possible to evaluate the LDL receptor status of the liver by sequential scintiscans. We conclude that this approach is useful to study the kinetics of the LDL metabolism externally, in other organ compartments and systems as well.

2. MATERIALS AND METHODS

Sodium [^{131}I]iodide with a specific activity of 10–20 Ci (370–740 GBq)/mg was purchased from Amersham-Buchler (Braunschweig, FRG). Cremophor E was obtained from BASF (Ludwigshafen,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

FRG). All other chemicals were purchased from Serva (Heidelberg, FRG).

2.1. *Animals, diets and drugs*

Female Sprague-Dawley rats weighing 250 g (10 weeks old) were used in all experiments. All rats were fed a normal rat chow diet. Beginning 5 days prior to [^{131}I]LDL scintigrams, 1 mg 17α-ethinyl estradiol was injected subcutaneously daily. It was dissolved first in 10 μl ethanol and then in 200 μl cremophor as a dilution mediator and in 800 μl phosphate-buffered saline (pH 7.2). Control rats received equal volumes of diluent.

2.2. *Low-density lipoprotein (LDL)*

Human LDL was isolated from plasma of normal rats by sequential ultracentrifugation as in [6]. The isolated LDL contained apoB as the only detectable band on SDS-PAGE. LDL was dialysed extensively against 0.15 M NaCl, 0.01% EDTA (pH 7) before use.

[^{131}I]LDL was labelled by a modification of the procedure of Mc Farlane [5], using low concentrations of chlorine water instead of ICl to generate free $^{131}I^{+}$. Excess radioiodine was rapidly removed by ultrafiltration using the Centricon system (Amicon, Witten, FRG). Protein concentrations were determined by the method of Lowry using albumin as a standard [7]. The specific activities for [^{131}I]LDL were 5000–7000 cpm/ng (10000–15000 dpm/ng = 4.5–7 μCi/μg). Lipid label was determined by butanol/acetone extraction as described by Bilheimer et al. [8]. The lipids contained less than 3% of the radioactivity in all experiments.

2.3. *In vivo studies*

Under anaesthesia with halothane/N_2O/O_2 (1.5:40:60), rats were injected with various amounts of [^{131}I]LDL through a tail vein. The distribution of the radiolabelled LDL in the body was continuously monitored with a pinhole camera (Phogamma III, Nuclear Chicago) interfaced with a PDP 11/30, Digital (München, FRG) to monitor the data on magtape in list mode. Data recording was started 0.5 min before injection. Images were taken at selected times. Regions of interest were defined. Radioactivity in the heart region was taken as being representative for the circulating radioactivity. Time–activity curves over the liver and heart regions were averaged for statistical variation.

3. RESULTS

The treatment of rats with 17α-ethinyl estradiol results in a marked hypolipoproteinemia [9]. In our animals we saw a fall in plasma cholesterol from 60 μg/dl before treatment to less than 6 μg/dl on day five.

3.1. *Perfusion scintiscans of the rat liver*

Since the application of high doses of 17α-ethinyl estradiol might influence the plasma space and the perfusion kinetics of the liver, in a series of control experiments in three normal rats and 3 rats pretreated with 17α-ethinyl estradiol, 80 μg [^{131}I]albumin (300–500 μCi) was injected intravenously to monitor perfusion of the organ compartments. As shown in fig.1, the distribution kinetics of [^{131}I]albumin in the liver and heart region of normal rats and rats pretreated with 17α-ethinyl estradiol obtained before injection of [^{131}I]LDL did not differ significantly. Rates of removal of ^{131}I from the circulating compartment were not affected (fig.1).

3.2. *Uptake and binding of [^{131}I]LDL by the rat liver receptors*

As expected, shortly after intravenous application of the [^{131}I]LDL, the radioactivity was detectable in organs with large blood flow, e.g., the heart and the liver. Within 20 s after injection, radioactivity reached a maximum in the heart region, then constantly decreased in the 17α-

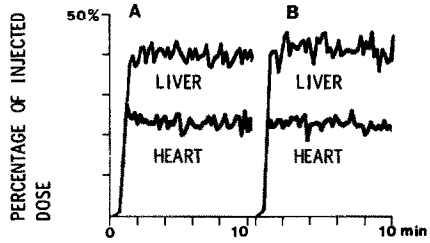

Fig.1. Time–activity curves in the liver and heart region in a normal rat and a rat treated with 17α-ethinyl estradiol 0–10 min after intravenous bolus injection of 80 μg [^{131}I]albumin = 300 μCi. Profiles of (A) an untreated (= normal) rat, (B) a rat after treatment with 17α-ethinyl estradiol (see section 2).

ethinyl estradiol-treated rat. Such a decay was not seen in the normal rat (fig.2). In contrast, in normal rats radioactivity reached an equilibrium in the plasma. In the estradiol-treated rat, simultaneously to the decay of [^{131}I]LDL in the heart region, 50% of the plasma LDL was cleared after 20 min as compared to less than 11% in the untreated rat. Thus the heart region represents a good estimate of the circulating radioactivity.

The time–activity curves in the liver region demonstrate that the hepatic uptake of [^{131}I]LDL is markedly affected by the estradiol pretreatment.

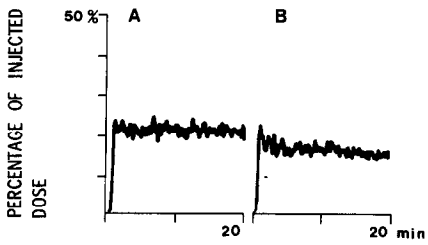

Fig.2. Time–activity curves in the heart region of a normal rat and a rat pretreated with 17α-ethinyl estradiol after intravenous bolus injection through a tail vein of 50 μg ^{131}I-labelled human LDL (400 μCi) from 0 to 20 min. Profiles from the heart region of (A) an untreated rat, (B) a rat after pretreatment with 17α-ethinyl estradiol (see section 2).

The removal of [^{131}I]LDL in estradiol-treated rats from the plasma compartment could be attributed largely to the uptake of the radioligand by the liver. A representative pair of hepatic time–activity profiles from a control and an estradiol-treated rat are shown in fig.3. The curves corrected for perfusion demonstrate a rapid and constant increase of [^{131}I]LDL trapping in the liver region of the estradiol-treated rat, binding 25% of the injected radioactivity 20 min after injection. In contrast, in the experiments with 3 control rats the radioactivity in the liver region averaged 3–7% of

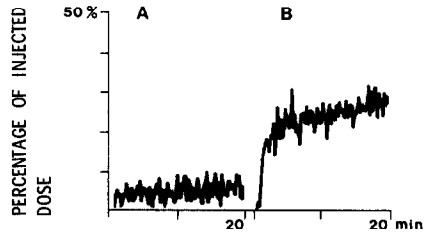

Fig.3. Time–activity curves in the liver region of a normal rat and a rat pretreated with 17α-ethinyl estradiol after injection of a bolus of 50 μg [^{131}I]LDL (400 μCi) through a tail vein from 0 to 20 min. Profiles are corrected for perfusion, thus representing accumulation of radioactivity due to binding to the organ compartment. Profiles from the liver region of (A) a normal rat, (B) a rat pretreated with 17α-ethinyl estradiol (see section 2).

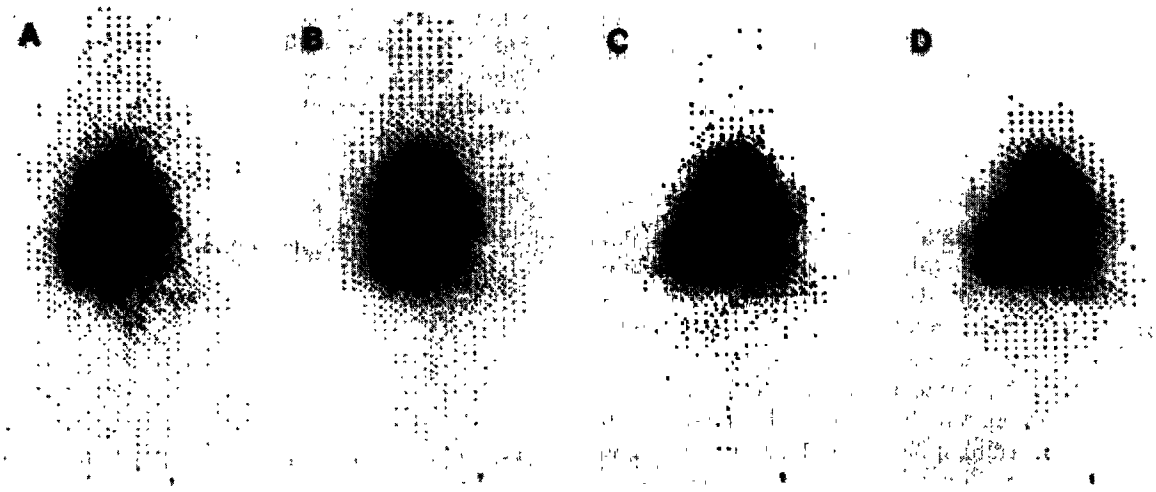

Fig.4. Sequential scintiscans of a normal rat and a rat pretreated with ethinyl estradiol. (A,B) 5 and 20 min after bolus injection of 50 μg ^{131}I-labelled human LDL (400 μCi) through a tail vein into a normal rat. (C,D) 5 and 20 min after bolus injection of 50 μg ^{131}I-labelled human LDL with the same specific activity through a tail vein of a rat injected daily with 5 mg/kg 17α-ethinyl estradiol intraperitoneally and an actual plasma cholesterol level of 3 μg/dl.

the injected dose. The differences in the LDL uptake in the liver compartments in the normal and estradiol-treated rat can be demonstrated nicely in total body images. Sequential scintiscans from normal and pretreated rats after injection of [^{131}I]LDL differentiate the liver and visualize clearly the difference in the liver uptake by the normal and estradiol-treated rats (fig.4).

Finally, an attempt was made to investigate whether the presence of unlabelled endogenous LDL would result in an effective competition with the injected [^{131}I]LDL. By preinjection of varying amounts of up to 4 mg unlabelled LDL, 10 min before the [^{131}I]LDL was applied, it was possible to increase the LDL plasma levels even in the estradiol-treated rat 2-fold above the plasma levels in normal rats. However, our experiments failed to demonstrate effective competition. Again, the hepatic accumulation of [^{131}I]LDL was rapid. Liver uptake was not affected at plasma LDL concentrations of 25, 100 and 160 μg/ml unlabelled plasma LDL-protein. This is in very good agreement with earlier reports by others, who quantified the hepatic LDL uptake in rats after killing and removal of the liver [1,4]. Therefore, we suggest that in the presence of physiological LDL plasma concentrations in the rat, the influence of competition in the presence of physiological endogenous LDL levels on the [^{131}I]LDL trapping activity in the liver is negligible.

4. DISCUSSION

External imaging of receptors for radioligands injected intravenously has been used to study the kinetics of ligand-receptor interaction in vivo in several systems [10–12]. We used this technique to follow the hepatic LDL receptor activity in the rat liver.

Based on the original observations of Kovanen et al. [1], we used normal rats and rats pretreated with α-ethinyl estradiol to demonstrate differences in LDL receptor-dependent uptake of LDL in this animal system by scintigraphy. It is apparent from our studies about the hepatic LDL uptake in vivo in normal rats and rats pretreated with 17α-ethinyl estradiol that sequential scintiscans and external recording of distribution kinetics of the radioligand are a very efficient approach to study the LDL interaction with the hepatic compartment in vivo. In confirmation of the results of Kovanen et al. [1], Chao et al. [2] and Koeltz et al. [4] we found that after 20 min, approx. 25–30% of the clearance of non-saturating amounts of LDL in the estradiol-treated rats can be attributed to a specific LDL trapping activity induced by 17α-ethinyl estradiol treatment. Some active receptors may also be demonstrated in the liver of normal rats which cannot be detected by our camera system. But under similar conditions, Kovanen et al. have shown that the hepatic LDL binding in normal rats is less than 10% of the liver of the estradiol-treated rat.

Since the pool size of endogenous LDL in the normal rat was greater than in the estradiol-treated rat, a possible influence of competition by endogenous lipoproteins in the normal rat could have affected the results. But neither the fractional catabolic rate nor the hepatic uptake kinetic was altered effectively in the presence of concentrations of unlabelled LDL as high as 160 μg/ml, which is 2-times the physiological levels of the normal rat. The failure to demonstrate competition at this concentration by endogenous LDL can be explained following the results of Kovanen et al. [1]. These investigators could calculate that half maximal concentration for LDL uptake in vivo in the estradiol-treated rat is 744 μg LDL-protein/ml, well above the concentration of the endogenous LDL. If this can be assumed – and our own experiment in the presence of high levels of endogenous LDL provides additional evidence – the data obtained by sequential scintiscans in the presence of physiological concentrations of endogenous LDL would reflect closely the in vivo LDL receptor activity. Thus, the use of LDL with high specific activities and a conventional gamma camera system to generate sequential images of LDL receptor compartments is a favorable approach to studying in vivo conditions.

ACKNOWLEDGEMENTS

We wish to thank J. Otto and H. Trojan for their excellent technical assistance in these studies and Mrs R. Stephan for typing the manuscript. This work was supported by a research grant to Dr Dresel from the Deutsche Forschungsgemeinschaft (Dr 161/1).

REFERENCES

[1] Kovanen, P.T., Brown, M.S. and Goldstein, J.L. (1979) J. Biol. Chem. 254, 11367–11373.
[2] Chao, Y., Windler, E., Chen, G.C. and Havel, R. (1979) J. Biol. Chem. 254, 11360–11366.
[3] Chao, Y., Jones, A.L., Hradek, G.T., Windler, E.E.T. and Havel, R.J. (1981) Proc. Natl. Acad. Sci. USA 78, 597–601.
[4] Koeltz, H.R., Sherrill, B.C., Turley, S.D. and Dietschy, J.M. (1982) J. Biol. Chem. 257, 8061–8072.
[5] Mc Farlane, A.C. (1948) Nature 182, 53.
[6] Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345–1353.
[7] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[8] Bilheimer, D.W., Eisenberg, S. and Levy, R.I. (1972) Biochim. Biophys. Acta 260, 212–221.
[9] Davis, R.A. and Roheim, P.A. (1978) Atherosclerosis 30, 293–299.
[10] Sodoyez, J.C., Sodoyez-Goffaux, F., Guillaume, M. and Merchie, G. (1983) Science 219, 865–867.
[11] Huettinger, M., Corbett, J.R., Schneider, W.J., Willerson, J.T., Brown, M.S. and Goldstein, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 7599–7603.
[12] Dresel, H.A., Friedrich, E., Via, D.P., Schettler, G. and Sinn, H. (1985) EMBO J., in press.