Volume 218, number 1, 77–80 FEB 04782 June 1987

Regulation of low density lipoprotein metabolism by 26-hydroxycholesterol in human fibroblasts

José L. Lorenzo, Maria Allorio, Franco Bernini, Alberto Corsini and Remo Fumagalli

Institute of Pharmacological Sciences, University of Milan, I-20133 Milan, Italy

Received 6 April 1987

Oxygenated derivatives of cholesterol are believed to play a role in cellular cholesterol homeostasis through the feed-back control of its biosynthesis. We report that 26-hydroxycholesterol inhibits the specific binding, uptake and degradation of ^{125}I-LDL in human fibroblasts. The effect is dose-dependent, and saturation kinetics indicates a reduction of LDL-binding sites with no effect on ligand affinity. The results support a possible role of 26-hydroxycholesterol, a physiological oxysterol, in the regulation of cellular cholesterol homeostasis.

26-Hydroxycholesterol; Oxysterol; LDL; LDL receptor; (Human fibroblast)

1. INTRODUCTION

Human fibroblasts in culture derive their cholesterol from endogenous biosynthesis and from receptor-mediated uptake of low density lipoproteins (LDL) [1]. Oxygenated sterols repress 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) activity [1–3], and one of them is reported to decrease the specific binding of LDL to plasma membrane [4] in cultured mammalian cells.

The question then arises as to whether it is an oxysterol, rather than cholesterol itself, that modulates these mechanisms of cholesterol supply [5]. 26-Hydroxycholesterol (cholest-5-ene-3β,26-diol) is present in human meconium [6] and serum [7], and occurs in healthy and diseased human aorta [8,9]. Since this oxysterol is reported to inhibit HMG-CoA reductase activity in a mammalian cell system [10], the possibility that it could also interfere with receptor-mediated LDL metabolism was explored. Cultured human fibroblasts were used as a cellular system.

Correspondence address: R. Fumagalli, Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, I-20133 Milan, Italy

2. EXPERIMENTAL

26(*S*)-Hydroxycholesterol and its 26(*R*) epimer were obtained from Research Plus (Bayonne, NJ). Its purity was assessed by chromatographic techniques [9]. Commercial heparin (Na salt) was a gift of Crinos SpA (Como, Italy). Na^{125}I (carrier-free; spec. act. 16.7 mCi/μg) in 0.1 M NaOH was purchased from Amersham (Amersham, England). Culture media, buffers, fetal calf serum and disposable material were obtained as previously reported [11].

2.1. *Cells*

Human skin fibroblasts were grown from explants of skin biopsies obtained from normolipidemic clinically healthy individuals. Cells were grown in monolayer as in [11] and used between the fifth and fifteenth passage.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

Table 1

Degradation of ^{125}I-LDL in human fibroblasts: effect of cholesterol and 26(*S*)-hydroxycholesterol

Sterol	^{125}I-LDL degradation (ng LDL protein/mg cell protein)
None	159.0 (452)
Cholesterol	
0.1 μM	175.2 (464)
2.0 μM	162.4 (470)
6.0 μM	120.5 (461)
25(*S*)-Hydroxycholesterol	
0.1 μM	172.8 (445)
2.0 μM	60.0 (460)
6.0 μM	7.0 (440)

In parentheses is given the mean content of total cellular protein (μg/dish)

2.2. *Lipoproteins*

LDL (*d* 1.019–1.063 g/ml) were isolated from plasma of clinically healthy normolipidemic volunteers by sequential preparative ultracentrifugation [12]. LDL were iodinated with ^{125}I by the monochloride procedure of McFarlane [13] as modified for lipoproteins [14], and sterilized by filtration (spec. act. 110–234 cpm/ng LDL protein). Lipoprotein-deprived serum (LPDS) was prepared by ultracentrifugation [11].

2.3. *Binding, uptake and degradation of ^{125}I-LDL*

All experiments were performed in a similar format. Confluent monolayers of cells were preincubated for 24 h at 37°C in F-11 medium containing 5% LPDS to induce LDL receptors [1], in the presence of 26-hydroxycholesterol at the concentrations indicated. Control dishes contained the same volume of ethanol (1% final concentration). After this time a fixed concentration (10 μg/ml of LDL protein: experiments reported in fig.1 and table 1) or increasing concentrations of ^{125}I-LDL (experiment reported in fig.2) were added to medium and the incubation was continued for a further 4 h.

Cell surface specific binding, uptake (binding + internalization) and degradation of ^{125}I-LDL were evaluated as reported [11]. Each experimenal point represents the average value of duplicate or triplicate incubations.

Cell viability was routinely assessed by trypan

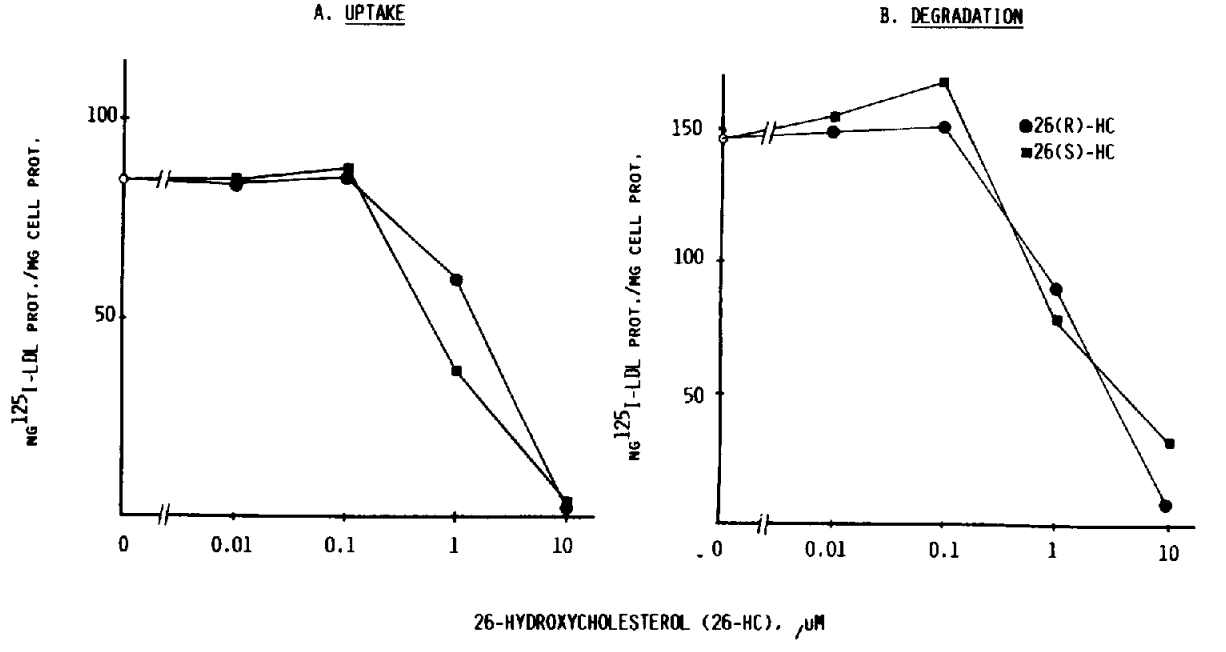

Fig.1. Uptake and degradation of ^{125}I-LDL by human fibroblasts: effect of 26(*R*)- and 26(*S*)-hydroxycholesterol.

blue exclusion and found to be greater than 90% under all experimental conditions. The protein content of cell monolayers, determined according to Lowry et al. [15], was not affected by incubation with 26-hydroxycholesterol: an example is reported in table 1.

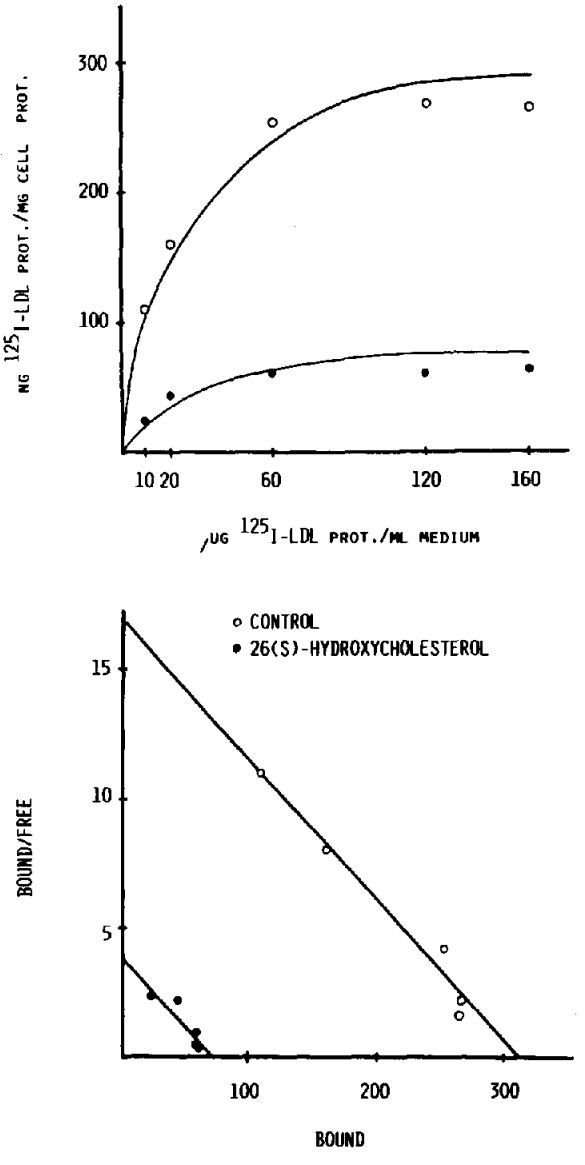

Fig.2. Cell surface binding of ^{125}I-LDL by human fibroblasts: effect of 26(*S*)-hydroxycholesterol; the lower panel represents the Scatchard analysis of the data. (o—o) $r = 0.97$, $p < 0.01$; (•—•) $r = 0.90$, $p < 0.05$.

3. RESULTS

26(*S*)-Hydroxycholesterol and its 26(*R*) epimer, tested at concentrations ranging between 0.01 and 10 μM, decreased cellular uptake (panel A) and degradation (panel B) of ^{125}I-LDL (fig.1); the inhibition was dose-dependent with no apparent difference between the two isomers.

Freshly crystallized cholesterol was less efficient in reducing cellular degradation of ^{125}I-LDL (table 1). In the experiment depicted in fig.2, the effect of a fixed concentration of 26(*S*)-hydroxycholesterol (10 μM) on specific cell surface binding of ^{125}I-LDL was evaluated at 37°C as a function of lipoprotein concentrations. To distinguish the amount of ^{125}I-LDL bound to the receptor from that which has been internalized, heparin (which selectively releases the ^{125}I-LDL bound to the cell surface receptor) was used, according to Goldstein et al. [16]. In the presence of the sterol, ^{125}I-LDL binding reached a plateau at lower values (upper panel). Scatchard analysis indicated that the oxysterol decreased the maximum amount of LDL bound at receptor saturation (317 vs 72 ng ^{125}I-LDL protein/mg cell protein), without affecting the binding affinity of the ligand (39 nM).

4. DISCUSSION

In recent years attention has been focussed on the biological properties of oxysterols. These compounds, in addition to other effects, suppress sterol biosynthesis in vivo as well as in cell cultures [5,16] by depressing the activity of HMG-CoA reductase [4,5,10]. The possibility that feed-back regulation of sterol biosynthesis could be brought about by an oxygenated sterol rather than by cholesterol itself has been considered [5]. 25-Hydroxy- and 26-hydroxycholesterols are among the most potent inhibitors of cholesterol biosynthesis, and the former was reported by Brown and Goldstein [4] to decrease receptor-mediated LDL uptake in human fibroblasts: the results of our experiments indicate that such an action is shared by 26-hydroxycholesterol.

25-Hydroxycholesterol is considered an autooxidation product of cholesterol [8,17], and its occurrence in vivo as part of a metabolic pathway is disputed [10,18]. In contrast, 26-hydroxycholesterol is the result of mitochondrial C27-steroid

26-hydroxylase [19,20], is an intermediate in the synthesis of bile acids [21], and is present in the serum of normal adults [7].

Esterman et al. [10] propose that 26-hydroxycholesterol has a selective biological role in the regulation of cholesterol biosynthesis. Our results show that this oxygenated sterol inhibits LDL pathway in human fibroblasts, thus also interfering with the extracellular source of cholesterol in mammalian cells in culture [1]. This effect involves a reduction of LDL-binding sites with no change in their affinity for the ligand, and occurs at concentrations of 26-hydroxycholesterol ($EC_{50} \sim 1\ \mu M$) not greatly different from those observed in human serum (0.23–0.64 μM) [7]. These results, in the light of the presence of 26-hydroxycholesterol in human LDL [7], support a possible regulatory role of this physiological oxysterol in cellular cholesterol homeostasis.

ACKNOWLEDGEMENTS

This research was partially supported by Ministero della Pubblica Istruzione and by Consiglio Nazionale delle Ricerche (Italian Government).

REFERENCES

[1] Goldstein, J.L. and Brown, M.S. (1977) Annu. Rev. Biochem. 46, 897–930.
[2] Gibbons, G.F. (1983) Biochem. Soc. Trans. 11, 649–651.
[3] Taylor, F.R., Kandutsch, A.A., Gayen, A.K., Nelson, J.A., Nelson, S.S., Phirwa, S. and Spencer, T.A. (1986) J. Biol. Chem. 261, 15039–15044.
[4] Brown, M.S. and Goldstein, J.L. (1975) Cell 6, 307–316.
[5] Kandutsch, A.A., Chen, H.W. and Heiniger, H.J. (1978) Science 201, 498–501.
[6] Lavy, U., Burstein, S. and Javitt, N.B. (1977) J. Lipid Res. 18, 232–238.
[7] Javitt, N.B., Kok, E., Burstein, S., Cohen, B. and Kutscher, J. (1981) J. Biol. Chem. 256, 12644–12646.
[8] Van Lier, J.E. and Smith, L.L. (1967) Biochemistry 6, 3269–3278.
[9] Fumagalli, R., Galli, G. and Urna, G. (1971) Life Sci. 10, 25–33.
[10] Esterman, A.L., Baum, H., Javitt, N.B. and Darlington, G.J. (1983) J. Lipid Res. 24, 1304–1309.
[11] Corsini, A., Granata, A., Fumagalli, R. and Paoletti, R. (1986) Pharmacol. Res. Commun. 18, 1–16.
[12] De Lalla, O.F. and Gofman, J.W. (1954) Methods Biochem. Anal. 1, 459–478.
[13] McFarlane, A.S. (1958) Nature 182, 53.
[14] Bilheimer, D.W., Eisemberg, S. and Levy, R.I. (1972) Biochim. Biophys. Acta 260, 212–221.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Goldstein, J.L., Basu, S.K., Brunschede, G.Y. and Brown, M.S. (1976) Cell 7, 85–95.
[17] Van Lier, J.E. and Smith, L.L. (1970) J. Org. Chem. 35, 2627–2632.
[18] Saucier, S.E., Kandutsch, A.A., Taylor, F.R., Spencer, T.A., Phirwa, S. and Gayen, A.K. (1985) J. Biol. Chem. 260, 14571–14579.
[19] Oftebro, H., Björkhem, I., Skrede, S., Schreiner, A. and Pedersen, J.I. (1980) J. Clin. Invest. 65, 1418–1430.
[20] Skrede, S., Björkhem, I., Kvittingen, E.A., Buchmann, M.S., Lie, S.O., East, C. and Grundy, S. (1986) J. Clin. Invest. 78, 729–735.
[21] Anderson, K.E., Kok, E. and Javitt, N.B. (1972) J. Clin. Invest. 51, 112–117.