Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 43-49

EFFECT OF MANIPULATION OF PHOSPHOLIPID POLAR HEAD GROUP ON LOW DENSITY LIPOPROTEIN METABOLISM IN HUMAN CULTURED FIBROBLASTS

C. Mazière, J.C. Mazière, L. Mora, M. Auclair, S.Salmon and J. Polonovski

Faculté de Médecine Saint Antoine, and U.A. 524 du C.N.R.S. 27 rue Chaligny 75012 Paris, France

Received March 14, 1986

SUMMARY: Modification of phospholipid polar head group was achieved by growing human cultured fibroblasts in medium devoid of serum and supplemented with N-methyl ethanolamine or N, N-dimethylethanolamine during 48 h. The corresponding phospholipids accounted for approximately 45 % of total phospholipids.

Whereas low density lipoprotein (LDL) binding was unaffected, LDL internalization and degradation appeared to be markedly reduced in the presence of N-methylethanolamine. N,N-dimethylethanolamine had no effect on the three studied parameters. These results emphasize the importance of phospholipid polar head group in LDL processing by receptor-mediated endocytosis.

The LDL receptor are located at specific regions of the cell surface: the so-called coated pits (1,2), which are clathrin-coated invaginations of the plasma membrane. The receptor-mediated endocytosis of the LDL molecules involves several membrane fusion steps. Coated pits give rise to coated vesicles (3), whose role is to deliver the LDL particles to lysosomes. While the LDL molecules are targeted to lysosomes where degradation occurs, the receptor itself is recycled back to the plasma membranes (4).

Phospholipids, as major components of the cellular membranes, might play an important role in intracellular transport and processing of LDL molecules. Modification of the fatty-acid composition of phospholipids can be achieved by growing cells in

ABBREVIATIONS : SM : sphingomyelin, PE : phosphatidyl-ethanolamine, PC : phosphatidyl-choline, PI : phosphatidyl-inositol, PME : phosphatidyl-monomethylethanolamine, PDE : phosphatidyl-dimethylethanolamine, LDL : low density lipoprotein, Hepes : N2-hydroxy-ethylpiperazine-N'2 ethanesulfonic acid.

0006-291X/86 $1.50

medium containing specific fatty acids (5). Using this procedure, Gavigan and Knight (6) reported that enrichment of culture medium with the polyunsaturated linoleic acid was associated with an increase in LDL degradation by cultured fibroblasts.

Alternatively, the phospholipid polar head group of cultured cells can be manipulated by supplementing the medium with choline analogues such as N-methylethanolamine or N,N-dimethylethanolamine (7,8). In the current studies, the effect of modification of phospholipid polar head group on LDL binding, internalization and degradation was investigated.

MATERIALS AND METHODS

Cell culture: MRC5 (human foetal lung) fibroblasts, purchased from Biomerieux, were routinely grown in Dulbecco Minimum Essential Medium supplemented with 10 % foetal calf serum (Gibco) at 37 C, in a 5 % CO_2 atmosphere.

Modification of phospholipid polar head group was performed by growing cells in medium devoid of serum, supplemented with Hepes buffer 10 mM, serum substitute Ultroser G (IBF France), and 80 to 200 µg/ml of choline or choline analogues (Sigma). The pH of the medium was adjusted to 7.4.

Determination of phospholipid composition: Cells were grown during 48 h in the presence of 50µCi/ml ^{32}P sodium orthophosphate 20 mCi/mg, CEA France, and the choline analogues. After harvesting, an aliquot of the cell suspension was applied on silica gel plate and analysed by bidimensional chromatography (9), first migration chloroform/methanol/H2O 65/25/4 v/v, second migration tetrahydrofuran/methanol/methylal/NH4OH 2N 40/20/20/4 v/v. After autoradiography, the phospholipid spots were cut out and counted in an Intertechnique instrument.

LDL binding, internalization and degradation : LDL was prepared from human serum by the method of Havel et al (10), and labeled according to Bilheimer et al (11), using ^{125}I Na (Amersham, 13-17Ci/mg). The specific radioactivity was about 200-300 dpm/ng.

After supplementation with choline analogues, cells were washed and LDL processing studied according to Goldstein et al (12), using 10µg/ml ^{125}I-LDL. Protein determination was done by the method of Lowry et al (13). Results are expressed in ng LDL/mg cell protein.

RESULTS

The phospholipid composition of MRC5 human fibroblast after 48h growth in the presence of choline analogues is shown in

Table I: Phospholipid composition of human fibroblasts MRC5 grown in medium containing choline or choline analogues

Addition	SM	PC	PI	PE	PME	PDE
None:	13.6±1.5	61.1±2.3	7.7±0.9	17.6±1.9	n.d.	n.d.
Eth :	13.4±1.8	55.9±2.5	8.3±0.6	22.4±2.1	n.d.	n.d.
MME :	13.9±1.7	37.3±1.8	7.1±0.5	n.d.	41.7±2.7	n.d.
DME :	14.1±1.1	27.1±2.1	7.0±0.7	4.3±0.4	n.d.	47.5±2.6
Chol:	13.5±1.7	62.3±3.5	7.2±0.8	17.0±1.7	n.d.	n.d.

Cells were incubated during 48h in Dulbecco Minimum Essential Medium supplemented with Hepes 10 mM, serum substitute Ultroser G, and 200 µg/ml ethanolamine (Eth), choline (Chol), monomethylethanolamine (MME) or dimethylethanolamine (DME), and 50 µCi/ml ^{32}P sodium orthophosphate. Radioactive phospholipids were then separated by bidimensional thin layer chromatography. Mean of 3 determinations ± s.d. n.d.: not detectable.

Table I. Supplementation with ethanolamine induced a slight increase in PE proportion, accompanied by a slight decrease in PC proportion. Changes in phospholipid composition following addition of N-monomethylethanolamine and N,N-dimethylethanolamine were quite noteworthy : the corresponding phospholipids, PME and PDE, accounted for 42 % and 48 % of total phospholipids respectively, thus representing the majority of cellular phospholipids. These modifications were accompanied, as expected, by a decrease in PC and PE proportions. By contrast, supplementation with choline yield no significant effect on the phospholipid composition of the studied cell line. It is of note that the amounts of minor phospholipids, such as SM or PI, were not affected by choline analogues supplementation. Further, no modification of cell morphology or adhesiveness occurred during the course of experiments

Figure 1 illustrates the effect of phospholipid polar head group modification on LDL binding, internalization and degrada-

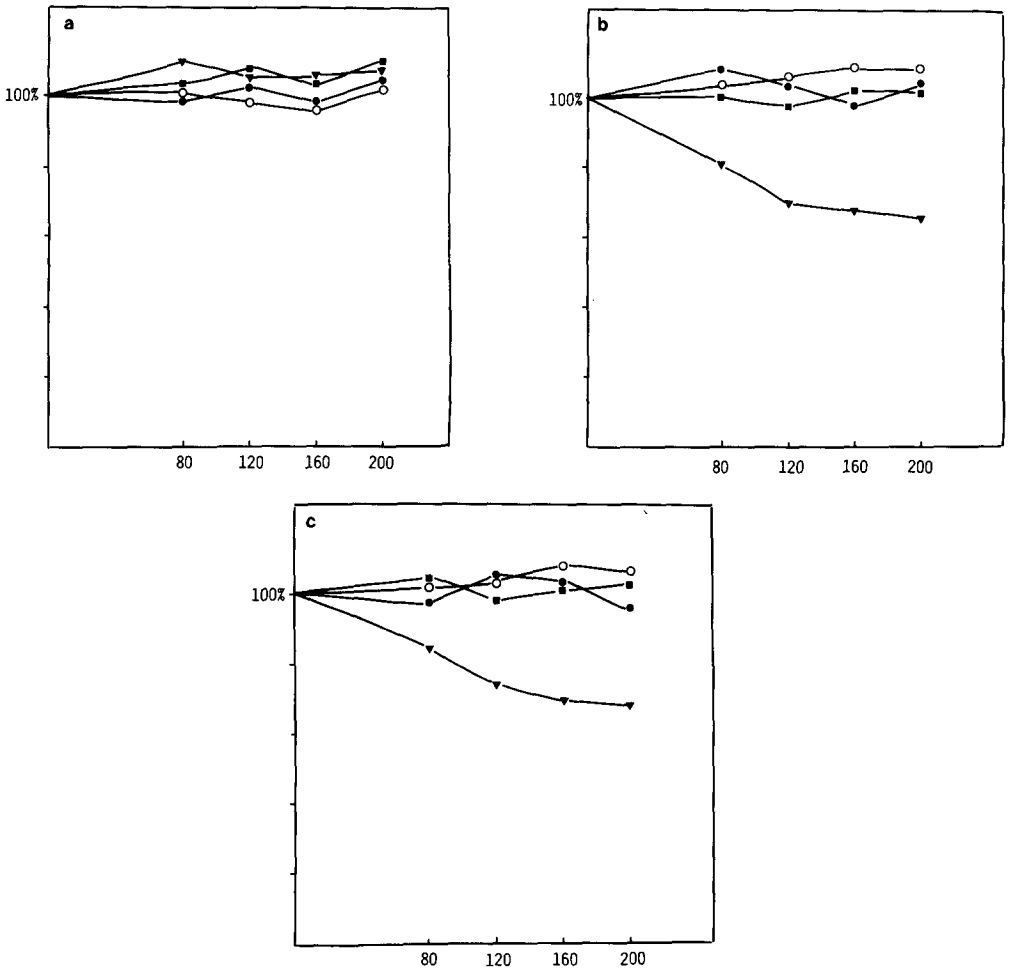

Figure 1 : Effect of manipulation of phospholipid polar head group on low density lipoprotein binding (1a), internalization (1b) and degradation (1c). Cells were grown during 48 h in medium supplemented with Hepes 10 mM, serum substitute Ultroser G and the indicated concentration of ethanolamine (●), monomethylethanolamine (▼), di-methylethanolamine (■) or choline (○) ^{125}I-LDL binding, internalization and degradation were then measured as described (12). Results are expressed in percentages of control. Mean of 3-5 determinations.

tion of LDL. It appeared that LDL binding, monitored at 4[C, is not dependent upon phospholipid polar head group. However, at 37[C, both LDL internalization and degradation are decreased in the presence of N-monomethylethanolamine, and thus with a dose-dependent effect. A maximum of 30-35 % decrease was obtained with 160-200 µg/ml of N-monomethylethanolamine. This effect appeared to be specific since supplementation with other analogues, espe-

cially with N,N-di-methylethanolamine, induced no effect on LDL processing by fibroblasts.

DISCUSSION

In the current studies, it is demonstrated that modification of phospholipid polar head group of cultured human fibroblasts can be achieved by supplementation with choline analogues. Similar results have been obtained with LM mouse fibroblasts (7,8), with chick embryo fibroblasts (14) or with rat C-6 glial cells (15). The possibility of manipulation of phospholipid polar head group of human cultured cells gives a good model for investigation of the activity of membrane-linked enzymes or receptors, in relation to their lipid environment.

Modification of phospholipid polar head group had no significant effect on LDL binding by human fibroblasts. It is therefore reasonable to assume that phospholipids play a minor role in the recognition of the LDL molecule by the specific receptor.

By contrast, LDL internalization and degradation appeared to be inhibited by enrichment of cellular membranes with PME. The fact that degradation is approximately reduced to the same extent than internalization indicates that most probably, LDL transport from membranes to lysosomes is impaired whereas the lysosomal degradation function is unaffected.

The mechanism whereby PME inhibits LDL intracellular transport remains unclear at the present time. One hypothesis might be that modification of membrane microviscosity could, in part, be responsible of this phenomenon, but different authors (16,17) reported that modification of cellular phospholipid composition did not modify the fluidity of cultured cells. Most probably, enrichment with PME induces some modification of the membrane structure, with reorganization of membrane constituants, so that mem-

brane invagination to coated pits, coated vesicles and endosomes might be inhibited.

Schroeder (18) demonstrated that alteration of the phospholipid composition of LM mouse fibroblasts, either by supplementation with N-methylethanolamine, NN-di-methylethanolamine or ethanolamine, correlated with a depression in polystyrene bead phagocytosis and horseradish peroxidase pinocytosis. It must be noted that phagocytosis and pinocytosis are non specific process, which do not involve the presence of specific receptors, coated pits and coated vesicles, as is the case in LDL endocytosis.

Thus, the inhibitory effect of PME on LDL uptake might be be ascribed to its action either on the budding of coated pits to coated vesicles, on the biogenesis or function of endosomes, or finally on the fusion of endosomes with lysosomes where degradation is achieved.

ACKNOWLEDGMENT

We thank Miss L. Berland for typing the manuscript.

REFERENCES

1. Anderson, R.G.W., Brown, M.S., and Goldstein, J.L., (1977), Cell, 10, 351-364.
2. Handley, D.A., Arbeeny, C.M., Witte, L.D., and Chien, S., (1981), Proc. Natl. Acad. Sci. U.S.A., 78, 368-371.
3. Pearse, B.M.F., (1982), Proc. Natl. Acad. Sci. U.S.A., 79, 451-455.
4. Goldstein, J.L., Anderson, R.G.W., and Brown, M.S., (1979), Nature (London), 279, 679-685.
5. Spector, A.A., Kiser, R.E., Denning, G.M., Koh, S.M., and De Bault, L.E., (1979), J. Lip. Res., 20, 536-547.
6. Gavigan, S.J.P., and Knight, B.L., (1981), Biochim. Biophys. Acta, 665, 632-635.
7. Glaser, M., Ferguson, K.A., and Vagelos, P.R., (1974), Proc. Natl. Acad, Sci. U.S.A., 71, 4072-4076.
8. Engelhard, V.H., Esko, J.D., Storm, D.R., and Glaser, M., (1976), Proc. Natl. Acad. Sci., U.S.A., 73, 4482-4486.
9. Gray, G.M., (1967), Biochim. Biophys. Acta, 144, 519-524.
10. Havel, R.J., Eder, H.A. and Bragdon, J.H., (1955), J. Clin. Invest., 34, 1345-1353.
11. Bilheimer, D.W., Grundy, S.M., Brown, M.S. and Goldstein, J.L., (1983), Proc. Natl. Acad. Sci., U.S.A., 80, 4124-4128.

12. Goldstein, J.L., Dana, S.E., and Brown, M.S., (1974), Proc. Natl. Acad. Sci, U.S.A., 71, 4288-4292.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., (1951), J. Biol. Chem., 193, 265-275.
14. Hale, A.H., Pessin, J.E., Palmer, F., Weber, M.J.,and Glaser, M., (1977), J. Biol. Chem., 252, 6190-6200.
15. Finkel, R.S., and Volpe, J.J., (1979), Biochim. Biophys. Acta, 572, 461-471.
16. Schroeder, F., Holland, J.F., and Vagelos, P.R., (1976), J. Biol. Chem., 251, 6747-6756.
17. Mc Kenzie, R.C., and Brophy, P.J., (1984), Biochim. Biophys. Acta, 769, 357-362.
18. Schroeder, F., (1981), Biochim. Biophys. Acta, 649, 162-174.