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INHIBITORY EFFECT OF PROSTAGLANDIN E_2 ON CHOLESTEROL ESTER ACCUMULATION IN MACROPHAGES

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The effects of prostaglandin E_2 (PGE_2) on the metabolism of β-migrating very low density lipoprotein (β-VLDL) in rat peritoneal macrophages were investigated. When cultured in vitro, macrophages apparently incorporated labelled β-VLDL time-dependently until 12h, when the incorporation appeared to reach a plateau. Labelled cholesterol ester in the macrophages increased rapidly until 2h of incubation, did not change from 2 to 6h, and then increased markedly until 28h. Labelled free cholesterol in the macrophages increased until 12h and then decreased until 28h. Exogenously added PGE_2 ($10^{-7} \sim 10^{-5}$ M) inhibited the accumulation of labelled cholesterol ester between 6h and 28h with or without increasing the level of labelled free cholesterol. These results suggest that PGE_2 prevented cholesterol ester accumulation by stimulating either hydrolysis of cholesterol ester or excretion of free cholesterol.

Macrophages may be converted to the foam cells commonly observed in intimal atheromatous lesions (1). Macrophages take up abnormal lipoproteins such as β-migrating very low density lipoprotein (β-VLDL) (2) and acetylated low density lipoprotein (3) via high affinity binding sites. The cholesterol ester in these lipoproteins taken up by macrophages is first hydrolyzed in lysosomes to free cholesterol, and the latter is either reesterified or released into the extracellular space (4),(5). This series of reactions has been named the scavenger pathway.

Abbreviations used: Prostaglandin E_2, PGE_2; β-migrating very low density lipoprotein, β-VLDL; acyl-CoA:cholesterol acyltransferase, ACAT; Dulbecco's modified Eagles medium, DME.

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Prostaglandins affect cholesterol ester metabolism in the aorta and in cultured aortic smooth muscle cells. These are reports that PGE_1 and E_2 inhibited (6),(7) or did not affect (8),(9) cholesterol esterase activity, that PGE_2 inhibited acyl-CoA: cholesterol acyltransferase (ACAT) activity (6),(9),(10), and that PGI_2 stimulated cholesterol esterase activity but had no effect on ACAT activity (8),(9). These reports show that prostaglandins are closely linked to lipoprotein metabolism. However, the role of prostaglandins in lipoprotein metabolism in macrophages is unknown. Since the main prostaglandin synthesized in macrophages is PGE_2, we studied its effect on β-VLDL metabolism in rat peritoneal macrophages.

Materials and methods

Culture of macrophages: Male albino Wistar rats weighing 300-350g were treated intraperitoneally with 3ml of 3% aqueous thioglycollate (Sanko Junyaku Co., Tokyo) solution. Three days later, their peritoneal cells were harvested in saline as reported (3). The cells from 2 rats collected by centrifugation at 400xg for 5min were combined and washed once with 20ml of Dulbecco's modified Eagle's medium (DME). The cells were then resuspended in 12ml of DME containing 10% fetal bovine serum (culture medium), and 1ml of this cell suspension was seeded into a Petri dish (35x10mm) and incubated for 2h in a CO_2 incubator. Then the dish was washed three times with 2ml of DME to remove non-adherent cells. The adherent cells were incubated for 24h with 1ml of culture medium and then used for experiments with medium change. The culture method was described in detail previosly (11). Treatment of macrophages: Macrophages were treated with 120μg protein of labelled β-VLDL or/and various amounts of PGE_2 (Ono Pharmaceutical Co., Osaka) in 1ml of culture medium and incubated for 1 to 28 h. Then the dishes were washed 5 times as reported (11). The lipids of cells were extracted, free cholesterol and cholesterol ester were separated as reported (11) and their radioactivities were counted. Preparation of labelled β-VLDL: A Japanese white rabbit weighing 1.5kg was given diet containing 1% cholesterol and 0.5% cholic acid for 2 months as reported (12). Then blood was collected and centrifuged at a density 1.063 for 24h. The upper layer was further subjected to zonal ultracentrifugation and β-VLDL was separated (13). β-VLDL was labeled with cholesteryl-1,2,6,7-^{3}H(N) linoleate (80Ci/mmol; New England Nuclear, Boston, MA) by the method of Fielding et al. (14). The typical labelled β-VLDL contained 906mg/dl of cholesterol, 109mg/dl of triglyceride, 5.9mg/ml of protein, and 3.42×10^7dpm/ml (5.80×10^6dpm/mg protein) of [^{3}H]β-VLDL.

Results

Effect of incubation time on [^{3}H] β-VLDL metabolism (control culture, Fig. 1)

Macrophages were incubated with [^{3}H] β-VLDL for 1-28h. The apparent uptake of [^{3}H] β-VLDL total cholesterol increased time dependently until 12h, but not subsequently. Cholesterol ester accumulation increased time-dependently until 2h, remained steady from 2h to 6h, and increased again time-dependently from 6h to 28h. Free cholesterol accumulation increased time-dependently until 12h and then decreased until 28h.

Effects of PGE_2 on [^{3}H] β-VLDL metabolism (Fig. 2)

Macrophages were incubated with 10^{-6} M PGE_2 under otherwise similar conditions to those for Fig. 1. Comparison of results with those in Fig. 1 showed that PGE_2 decreased the apparent total cholesterol uptake especially after long incubation (20h and 28h). This decrease was attributed to decrease in the level

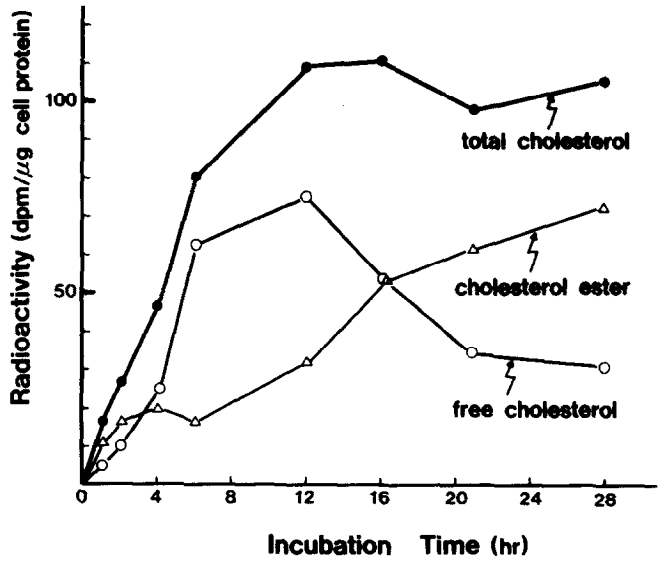

Figure 1: Effect of incubation time on [^{3}H] β-VLDL metabolism Macrophages were incubated with 120μg of [^{3}H] β-VLDL in 1ml of culture medium for 1-28h. At each incubation time cellular lipids were extracted and separated and the radioactivities of the different fraction were counted. Points are means of for duplicate cultures.

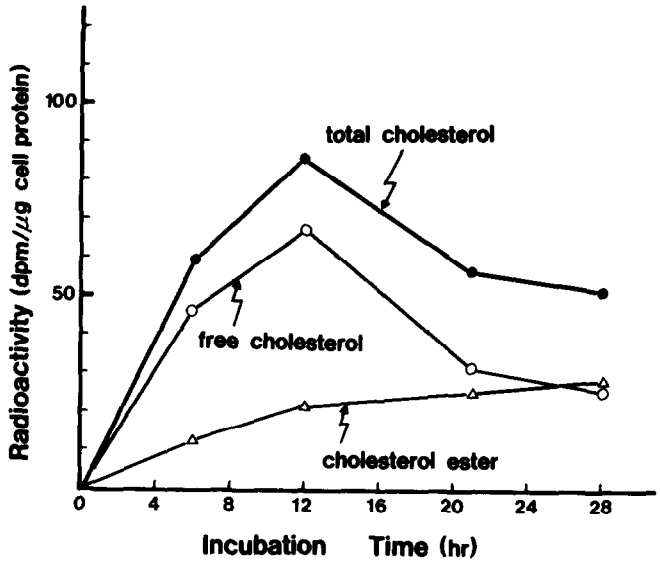

Figure 2: Effect of PGE_2 on [^{3}H]β-VLDL metabolism as a function of the incubation time. Conditions were as for Fig. 1 except that cultures contained 10^{-5} M PGE_2.

of cholesterol ester, which accumulated only gradually until 28h. But at 28h, free cholesterol accumulation was only slightly less in the presence of PGE_2 than that in its absence (Fig. 1).

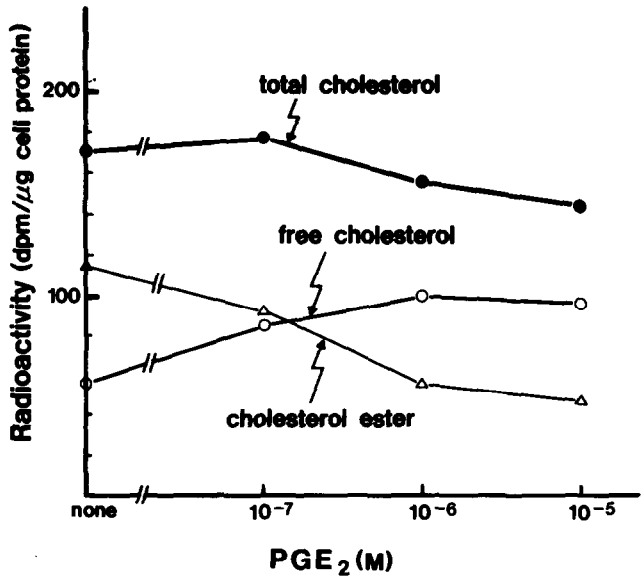

Figure 3: Dose dependence of effects of PGE_2 on [^{3}H]β-VLDL metabolism. Conditions were as for Fig. 1, but different cultures were used from those for Figs 1 & 2. The incubation time was 24h.

Dose-dependent effects of PGE_2 on [^{3}H]β-VLDL metabolism (Fig. 3)

As PGE_2 had a great effect after a long period of incubation, we examined the dose-dependence of its effects after an incubation period of 24h. In this experiment, total cholesterol accumulation was decreased slightly only by 10^{-6} and 10^{-5} M PGE_2. However, cholesterol ester accumulation was inhibited dose-dependently by PGE_2, with reciprocal increase in free cholesterol accumulation.

Discussions

The present study showed that exogenously added PGE_2 caused dose- and time-dependent inhibition of cholesterol ester accumulation in macrophages incubated with β-VLDL (Figs. 1,2,3). Ho et al. (5) reported that free cholesterol was lost from macrophages when the medium contained cholesterol acceptors such as high density lipoproteins, whole serum, the bottom fraction, erythrocytes, or thyroglobulin. Since culture medium contained some of these components, the results in Figs. 1 to 3 may not simply indicate the uptake of lipoprotein, but may reflect the over all results of uptake, metabolism (hydrolysis and reesterification), and excretion of the lipoprotein lipids.

As cholesterol ester is not excreted into the extracellular space, the [^{3}H]cholesterol ester in macrophages consisted of both the cholesterol ester taken up directly and that formed by reesterification in the cells. The [^{3}H]cholesterol ester that increased in control cultures after incubation for 12h to 28h was probably formed by reesterification because increase in [^{3}H] cholesterol ester was reciprocal with decrease in [^{3}H]free cholesterol. Although macrophages retained the capacity to hydrolyze cholesterol until 12h, the cells may have lost this capacity after 12h for some reason. If so, the results obtained for control cultures show that the excretion of free cholesterol

from the cells was not significant, and the plateau of [^{3}H]total cholesterol accumulation at 12h in these cultures indicates that uptake of lipoprotein ceased. However, in the cultures with PGE_2 (Fig. 2) the decrease in [^{3}H]free cholesterol was much greater than the increase in [^{3}H]cholesterol ester. This finding strongly suggests that free cholesterol was secreted into the medium, causing decrease in total cholesterol in the macrophages.

There are two possible sites of action of PGE_2. First, PGE_2 may stimulate cholesterol ester hydrolysis either in acidic or neutral conditions. Increased cholesterol ester hydrolysis would lead to increase in the intracellular level of free cholesterol and excretion of the latter into the medium. Second, PGE_2 may stimulate the excretion of free cholesterol into the medium, which would result in decrease in the free cholesterol content, resulting in inhibition of reesterification of cholesterol. Whichever the mechanism, the present results indicate that macrophages lose the capacity to metabolize β-VLDL very rapidly with consequent accumulation of cholesterol ester and that in the presence of prostaglandin E_2 they retain this capacity for longer and so show less lipid accumulation. Our data suggest that treatment of macrophages with arachidonic acid may enhance their ability to produce prostaglnadins and inhibit foam cell formation in atheromatous lesions.

References

1. Gerrity, R.G., Naito, H.K., Richardson, M., and Schwarts, C.J. (1979) Am.J.Pathol. 95:775-786.
2. Goldstein, J.L., Ho, Y.K., Brown, M.S. Innerarity, T.L., and Mahley, R.W. (1980) J.Biol.Chem. 255:1839-1848.
3. Goldstein, J.L., Ho, Y.K., Basu, S.K., and Brown, M.S.(1979) Proc.Natl.Acad.Sci.USA 76:333-337.
4. Brown, M.S., Goldstein, J.L., Krieger, M., Ho, Y.K., and Anderson, R.G.W. (1979) J.Cell Biol. 82:597-613.
5. Ho, Y.K., Brown, M.S., and Goldstein, J.L. (1980) J.Lipid Res. 21:391-398.
6. Subbiah, M.T.R. and Dicke, B.A. (1977) Atherosclerosis 27:107-111.
7. Subbiah, M.T.R. (1978) Atherosclerosis 29:487-495.

8. Hajjar, D.P., Weksler, B.B., Falcone, D.J., Hefton, J.M., Tack-Goldman, K., and Minick, G.R. (1982) J.Clin.Invest. 70:479-488.
9. Hajjar, D.P. and Weksler, B.B. (1983) J.Lipid Res. 24:1176-1185.
10. Berberian, P.A., Ziboh, V.A., and Hsia, S.L. (1977) Atherosclerosis 27:213-220.
11. Morisaki, N., Kanzaki, T., Fujiyama, Y., Osawa, I., Shirai, K., Matsuoka, N., Saito, Y., and Yoshida, S. (1985) J.Lipid Res. 26:930-939.
12. Morisaki, N., Shinomiya, M., Mizobuchi, M., Fujiyama, Y., Sasaki, N., Shirai, K., Matsuoka, N., Saito, Y., and Kumagai, A. (1982) Artery 11:225-237.
13. Patsch, J.R. (1975) Eur.J.Clin.Invest. 5:45-55.
14. Fielding, C.J. (1973) Biochim. Biophys. Acta 316:66-75.