Volume 236, number 1, 115–118 FEB 06175 August 1988

PAF-acether decreases low density lipoprotein degradation and alters lipid metabolism in cultured human fibroblasts

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Received 26 April 1988; revised version received 20 June 1988

A 24 h pretreatment of human cultured fibroblasts with PAF-acether (PAF) induced a decrease in LDL degradation and a correlative accumulation of undegraded LDL. LDL binding was not significantly affected. Sterol and triacylglycerol synthesis from sodium acetate was enhanced whereas phospholipid synthesis decreased. Oleic acid incorporation into cholesteryl ester was markedly inhibited, whereas incorporation into triacylglycerols was increased. A decrease in the percentage of phosphatidylcholine and an increase in the percentage of phosphatidylethanolamine were found using sodium [^{32}P]orthophosphate as precursor. These effects of PAF on LDL and lipid metabolism could be related to perturbations in membrane structure characteristics, leading to a delay in LDL delivery to lysosomes, and to modification of the activity of some key enzymes of lipid metabolism.

Low density lipoprotein; Platelet-activating factor; (Human fibroblast)

1. INTRODUCTION

PAF, or acetylglyceryl-ether-phosphorylcholine, is an ether phospholipid analog of phosphatidylcholine and was first described as a mediator of platelet aggregation during immunoglobulin E-induced anaphylaxis in the rabbit [1,2]. Subsequently, it was demonstrated that PAF is produced by a series of cell types (see [3] for review), especially by endothelial cells [4,5]. The increased adhesion and activation of both platelets and granulocytes at the endothelium surface might play a major role in the process of inflammation or in the formation of atherosclerotic plaques.

The role of the LDL receptor pathway of fibroblasts in the appearance and extent of atherosclerosis was well established by Brown and Goldstein [6,7], in that a decrease in LDL receptor number, as observed in familial hypercholesterolemia [8], leads to premature atherosclerosis. After binding to the cell surface, the LDL particles are internalized by means of coated vesicles and endosomes [9]. The LDL particles then reach lysosomes where degradation occurs. The liberated cholesterol in turn decreases endogenous cholesterol synthesis [10], activates cholesterol esterification [11] and inhibits the LDL receptor synthesis itself.

In the current studies, the effects of PAF on LDL and lipid metabolism were investigated in cultured human fibroblasts.

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Abbreviations: LDL, low density lipoprotein; PAF, platelet activating factor, PAF-acether

2. MATERIALS AND METHODS

2.1. *Materials*

PAF (DL-α-phosphatidylcholine, β-acetyl-γ-*O*-hexadecyl), lyso PAF (DL-α-lysophosphatidylcholine, γ-*O*-hexadecyl) and β-acetylphosphatidylcholine (L-α-phosphatidylcholine, β-acetyl-γ-oleoyl) were from Sigma, St. Louis, MO, USA. Sodium [2-^{14}C]acetate, 55 mCi/mmol, and sodium [^{32}P]orthophosphate, 20 mCi/mg, were from CEA, Saclay, France; [1-^{14}C]oleic acid, 52 mCi/mmol, and Na^{125}I, 13–17 Ci/mg, were from Amersham, Buckinghamshire, England. Dulbecco's modified minimum essential medium (MEM) with Earle's salts and fetal calf serum were from Gibco, Grand Island, NY. MRC5 human fetal lung fibroblasts were purchased from

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BioMérieux, France. The serum substitute, Ultroser G, was from Industries Biologiques Françaises, La Queue en Yvelines, France. Silica gel plates F 1500 were from Schleicher and Schull, Dassel, FRG.

2.2. *Cell culture*

Cells were cultured in 60 mm Nunc Petri dishes containing 2 ml Dulbecco's MEM supplemented with 20 mM Hepes buffer (pH 7.4), 100 units/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) fetal calf serum, at 37°C in a humidified atmosphere of 5% CO_2. Experiments were performed on confluent cells.

2.3. *LDL preparation and labeling*

LDL was prepared from normal human serum according to Havel et al. [12] and labeled according to Bilheimer et al. [13]. The specific radioactivity was 200–300 dpm/ng.

2.4. *Effect of PAF on LDL binding, internalization and degradation*

Cells were pretreated 24 h with PAF in ethanol solution (final concentration 0.5%) in medium supplemented with 2% Ultroser G for induction of LDL receptors. Cells were then washed 3 times, and LDL binding, internalization and degradation studied according to [14], using 10 μg protein/ml ^{125}I-LDL. Results are expressed in ng LDL/mg of cell protein. Protein determination was done by the Lowry method.

2.5. *Effect of PAF on lipid metabolism*

Cells were preincubated 24 h with PAF in medium supplemented with either 2% Ultroser G (maximum sterol synthesis) for sodium acetate incorporation or 10% fetal calf serum (induction of cholesterol esterification). Then radioactive precursors, sodium acetate, 40 μCi/ml, or oleic acid, 1 μCi/ml, resuspended in 0.2 mg/ml albumin were added. After a further 4 h incubation, cells were washed 3 times and lipid analysis performed by thin-layer chromatography after application of a cell suspension aliquot to silica gel plates [15]. The solvent system was hexane/diethyl ether/acetic acid (70:30:2, v/v) for neutral lipid analysis or chloroform/methanol/acetic acid/water (50:30:8:4, v/v) for phospholipid analysis. After autoradiography, the radioactive spots were cut out and counted by liquid scintillation with an Intertechnique instrument. Results are expressed in pmol precursor incorporated/mg cellular protein.

2.6. *Effect of PAF on phospholipid composition*

Cells were grown for 48 h in the presence of 10 μCi/ml sodium [^{32}P]orthophosphate, then further treated for 24 h with PAF (10^{-5} to 5×10^{-5} M) in the radioactive medium. After extensive washing, lipid analysis was performed by thin-layer chromatography as described above. Results are expressed in percentages.

Results were means of 4 or 6 experimental values ± SD. Statistical analysis was performed by the Mann-Whitney–Wilcoxon test.

3. RESULTS

The effects of PAF on LDL metabolism are presented in table 1. It can be noted that whereas LDL binding, measured at 4°C, was not modified after pretreatment of human fibroblasts with PAF, the cell-bound radioactivity measured at 37°C was increased in a dose-dependent manner to reach about 160% of control at 5×10^{-5} M PAF. In contrast, LDL degradation was inhibited by PAF especially at the highest concentration and accounted only for 65% of control. It is of note that, in the same range of concentrations, lyso PAF or β-acetylphosphatidylcholine did not significantly affect LDL internalization and degradation.

Table 1

Effects of platelet activating factor on LDL binding, uptake and degradation by cultured human fibroblasts

Addition	Membrane bound (4°C)	Intracellular (37°C)	Degraded (37°C)
Control	45 ± 4	192 ± 13	936 ± 91
PAF			
10^{-5} M	42 ± 5	240 ± 18[a]	898 ± 82
2×10^{-5} M	46 ± 4	257 ± 23[a]	861 ± 67
5×10^{-5} M	43 ± 3	303 ± 31[b]	618 ± 51[b]
Lyso PAF,			
5×10^{-5} M	n.d.	196 ± 11	951 ± 79
β-Acetylphosphatidylcholine,			
5×10^{-5} M	n.d.	193 ± 14	942 ± 71

Cells were pretreated 24 h with PAF, lyso PAF or β-acetylphosphatidylcholine in ethanol solution (final concentration of ethanol 0.5%) in lipoprotein-deficient Ultroser G supplemented medium for maximum induction of the LDL receptors. Binding, uptake and degradation were measured with 10 μg/ml ^{125}I-LDL. Results are expressed in ng LDL/mg cell protein. Means of 6 experimental values ± SD. [a] $p < 0.05$; [b] $p < 0.01$

Table 2

Effects of platelet activating factor on lipid synthesis in cultured human fibroblasts

Addition	pmol precursor/mg protein incorporated into		
	Sterols	Triacylglycerols	Phospholipids
Control	465 ± 52	1153 ± 91	7585 ± 571
PAF			
10^{-5} M	599 ± 58[a]	1983 ± 112[b]	6372 ± 523
2×10^{-5} M	744 ± 62[b]	2721 ± 189[c]	5690 ± 492[a]
5×10^{-5} M	837 ± 74[b]	4312 ± 319[c]	4552 ± 426[a]

Cells were pretreated 24 h with PAF in Ultroser G-supplemented medium (maximum sterol synthesis) before addition of sodium acetate, 40 μCi/ml, during 4 h. Means of 4 experimental values ± SD. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

Table 3

Effects of platelet activating factor on oleic acid incorporation into cholesteryl esters, triacylglycerols and phospholipids in cultured human fibroblasts

Addition	pmol precursor/mg protein incorporated into		
	Cholesteryl esters	Triacylglycerols	Phospholipids
Control	396 ± 32	2090 ± 193	2043 ± 158
PAF			
10^{-5} M	277 ± 28[a]	2404 ± 197	1798 ± 144
2×10^{-5} M	249 ± 19[b]	2822 ± 218[a]	1594 ± 135[a]
5×10^{-5} M	154 ± 13[c]	3971 ± 254[b]	1511 ± 97[a]

Cells were pretreated 24 h with PAF in 10% fetal calf serum-supplemented medium (induction of the enzyme acyl coenzyme A:cholesterol acyltransferase) before addition of oleic acid, 1 μCi/ml, during 4 h. Means of 4 experimental values ± SD. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

The effects of PAF on lipid synthesis were further investigated and results are given in table 2. It can be seen that sterol synthesis from sodium acetate was increased by PAF pretreatment, together with a dose-dependent effect (180% of control at 5×10^{-5} M). Other lipid metabolism was also affected: triacylglycerol synthesis was markedly enhanced (3- to 4-fold), whereas phospholipid synthesis was decreased about 40%.

Concerning cholesterol esterification studied by oleic acid incorporation into cholesteryl esters, the data from table 3 show that the esterification rate was diminished in the presence of PAF to about 40% of control at 5×10^{-5} M. In contrast, oleic acid incorporation into triacylglycerols was enhanced (about a 2-fold increase was noted with 5×10^{-5} M PAF), whereas incorporation into phospholipids was decreased by about 30%.

As it can be assumed that some of the observed effects of PAF on LDL and lipid metabolism could be related to alterations of membrane physicochemical characteristics, we thus investigated the effect of the mediator on phospholipid composition. Table 4 shows that 2×10^{-5} M PAF significantly decreased the percentage of phosphatidylcholine by about 20–25%, whereas that of phosphatidylethanolamine was increased by about 35%. In cells treated with 5×10^{-5} M PAF, a decrease in the percentage of sphingomyelin was also observed. Concerning the percentage of phosphatidylinositol, a tendency to increase was noted at the highest concentration, but this phenomenon did not appear to be significant after statistical analysis.

4. DISCUSSION

In this work, it was demonstrated that PAF, while not affecting LDL binding, increased the intracellular radioactivity when cells were incubated at 37°C with ^{125}I-LDL. However, as LDL degradation was decreased in the presence of PAF, the only explanation is that the mediator induces an accumulation of LDL, either on their way to lysosomes or within the lysosomes. It is of note that the formation of endosomes from coated vesicles and the fusion of endosomes with

Table 4

Effect of platelet-activating factor on phospholipid composition in cultured human fibroblasts

Addition	Percentage of			
	SM	PC	PI	PE
None	10.8 ± 1.2	58.1 ± 4.2	9.3 ± 1.5	21.8 ± 3.3
PAF				
10^{-5} M	11.6 ± 1.5	52.1 ± 5.3	10.9 ± 1.3	25.4 ± 3.8
2×10^{-5} M	10.3 ± 1.0	47.2 ± 3.4[a]	12.0 ± 1.7	30.5 ± 3.1[a]
5×10^{-5} M	8.2 ± 0.8[a]	45.1 ± 4.8[a]	12.7 ± 1.8	34.0 ± 5.4[a]

Cells were grown during 48 h in 10% fetal calf serum-supplemented medium containing 10 μCi/ml sodium [^{32}P]orthophosphate, 20 mCi/mg, and then further treated with PAF for 24 h in the presence of [^{32}P]orthophosphate. Means of 4 experimental values ± SD. [a] $p < 0.05$. SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine

lysosomes involve membrane movement and reorganization which might be modified by the presence of PAF, a phospholipid analog.

Concerning cholesterol metabolism, the mediator PAF induced a stimulation of sterol synthesis and an inhibition of cholesterol esterification. It is well known that the rate of sterol synthesis is under the control of the key enzyme hydroxymethylglutaryl coenzyme A reductase [16], whereas the rate of cholesterol esterification is determined by the activity of acyl coenzyme A:cholesterol acyltransferase [17]; both enzymes were localized in microsomal membranes and have been shown to be regulated by membrane microviscosity [18,19]. In this regard, one might consider the hypothesis of an intercalation of PAF within the membrane phospholipids leading to modifications of membrane structure and physicochemical characteristics, which in turn induce some perturbations in membrane-bound enzymatic activities. This hypothesis is further supported by the alterations in membrane phospholipid composition observed in PAF-treated cells (table 4). It is noteworthy that the main feature observed is the decrease in phosphatidylcholine, which is located in the external leaflet of the membrane, whereas phosphatidylethanolamine, located in the internal leaflet, was decreased.

The concentrations of PAF utilized in our cell system were relatively high. But it must be emphasized that PAF is considered as a local mediator and that local concentrations within cell membranes might be markedly higher than expected. Furthermore, it was recently demonstrated that preformed PAF is transported in blood bound to lipoproteins [20], and thus might be delivered to cells with great efficiency by means of the LDL receptor pathway.

This work demonstrates that besides the well admitted role of PAF in inflammation, this phospholipid mediator influences the LDL receptor pathway and cholesterol metabolism in cultured fibroblasts and hence might be involved in the pathogenic events leading to the formation of atherosclerotic plaques.

Acknowledgements: We acknowledge La Ligue Nationale Française contre le Cancer for financial support. We thank Miss Françoise Farchi for typing the manuscript.

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