Volume 269, number 1, 171–173 FEBS 08754 August 1990

Paf-acether-induced superoxide anion generation in human B cell line

Gerald Leca[1], Francine Joly[2], Aimé Vazquez[1], Pierre Galanaud[1] and Ewa Ninio[2]

[1]*Inserm U131 and Association Claude Bernard Research Center and* [2]*U200 32 rue des Carnets, 92140 Clamart, France*

Received 5 June 1990

Paf-acether (paf) and lyso phospholipids induced an oxydative burst on EBV-transformed B lymphocyte cell line. Superoxide anion formation measured by lucigenin-dependent chemiluminescence was dependent on both paf concentration and time-course of challenge. Paf C18:0 at 10 μM was more potent than its C16:0 analogue at the same concentration. Choline-containing phospholipids with 2-acyl (long chain) were inactive. The paf antagonists BN 52021 and WEB 2086 structurally unrelated to paf were inactive whereas paf structural analogue CV 3988 inhibited superoxide formation induced by paf and lysophospholipids. Such a phospholipid-induced oxydative burst in B cells might exert an effect in the numerous pathophysiological situations where large amounts of paf are produced by phagocytic cells.

B cell line (human); Paf-acether; Superoxide anion

1. INTRODUCTION

In a variety of cells including human lymphoblastoid B cells [1] paf-acether (paf, 1-*O*-hexa/octadecyl-2--acetyl-*sn*-glycero-3-phosphocholine; for references see review in [2]) acts via specific binding sites functionally coupled to phospholipase C-initiated phosphatidylinositol hydrolysis [3]. The specificity of paf binding is evidenced by displacement of radiolabeled paf with unlabelled paf or with specific paf antagonists [4]. Paf antagonists such as gingkolides [5] triazolobenzodiazepines [6] or structural analogues of paf [5,7] are equally very potent inhibitors of cellular paf effects [4] suggesting that the biochemical events initiated by paf are related to occupancy of the specific paf binding sites.

The expression of NADPH-oxidase and the potential for stimulus-triggered superoxide anions (O_2^- production) is one of the main features of phagocytes. Recently, several reports showed that human B lymphocytes were able to form superoxide anions and derived reactive oxygen radicals when stimulated with PMA or by cross-linking of surface immunoglobulins [8].

Paf, an extra- and intercellular mediator [2] that generates one of the earliest signals in the transmembrane transducing system [3] is also implicated in the superoxide formation in phagocytes and polymorphonuclear neutrophils [9,10]. Paf was also recognized to possess immunoregulatory functions in human cells [11], some of which might be mediated by superoxide ions generation in situ by either phagocytes and polymorphonuclear neutrophils or other undetermined cell types. We thus tested the hypothesis of paf involvement in the oxidative burst of EBV-transformed B lymphocyte cell line (EBV-BLCL).

Correspondence address: G. Leca, INSERM U131, 32, rue des Carnets, 92140 Clamart, France

2. MATERIALS AND METHODS

Synthetic C16:0 and C18:0 paf, 1-*O*-hexa/octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-*O*-hexa/octadecyl-2-acetyl-GPC), C16:0 and C18:0 lyso paf, 1-*O*-hexa/octadecyl-GPC, 1-*O*-hexadecyl-2-*N*-methylcarbamyl-GPC and 1-*O*-hexadecyl-2-palmitoyl-GPC were obtained from Novabiochem (Laufenfingen, Switzerland). Radiolabelled paf (1-*O*-[^{3}H]octadecyl, 80-120 mCi/mmol or *N*-methyl-[^{14}C], 58 μCi/mmol) and OCS scintillation liquid were from Amersham Int. (UK). Paf antagonists were gifts as noted: BN 52021 (Institut Henri Beaufour, Plessis Robinson, France), WEB 2086 (Boehringer Ingelheim, Ingelheim, FRG), CV 3988 (Takeda Chemical Ind., Osaka, Japan).

2.1. *B lymphocyte cell line establishment*

GL1 cell line was obtained after infection of normal B cells using B 95.8 supernatant (EBV-producing Marmoset lymphoblastoid cell line) as described in [12]. Cell surface marker analysis was performed using an Orthocytofluorograph (model 50 H) by using FITC-labelled F(ab')$_2$ fragments of rabbit anti-human IgG and IgM (Jackson Immunoresearch Lab., West Grove, PA, USA) and following FITC mouse monoclonal antibodies (Becton Dickinson, Mountain View, CA, USA): anti-Leu 12 (B cells), anti-Leu M3 (monocytes), anti-Leu 4 (T cells).

2.2. *Lucigenin-chemiluminescence measurements*

Chemiluminescence (CL) was measured using the luminometer (PICO-Lite, Packard 6100, Downers Grove, IL, USA) as follows: 200 μl of cell suspension (5×10^6 cells/ml of MEM medium) was transferred into a borosilicated tube and 25 μl of the agent to be tested was added. The lucigenin solution (25 μl, bis-*N*-methylacridinium nitrate; Sigma, St. Louis, MO, USA) was then added to a final concentration of 100 μM. The tubes were transferred into the counting chamber of the luminometer with the thermostat set at 37°C. Results are expressed in cpm after substraction of the background mean value.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

2.3. *[^{3}H]paf metabolism by EBV-BLCL*

Cells (5×10^6/ml of MEM Dulbecco medium) were incubated up to 20 min at 37°C with 10 μM [^{3}H]paf and then extracted with 4 vols of absolute ethanol. After centrifugation ($1500 \times g$ for 15 min, 20°C) supernatants were dried (40°C) under an air stream and the residues were further analysed by liquid chromatography [13] using [^{14}C]paf as an internal standard.

3. RESULTS AND DISCUSSION

Two molecular species of paf were used in this study: C18:0 and C16:0 paf. Both elicited superoxide formation in EBV-BLCL. The lucigenin-dependent chemiluminescence signal (reflecting O^{2-} formation) was dependent on both paf concentration and time-course of challenge. Paf C18:0 was more potent than its C16:0 analogue (Fig. 1). [^{3}H]paf (10 μM) added to the cells for at least 20 min at 37°C was not degraded since in two experiments 83% of radioactivity was recovered after liquid chromatography analysis as the single peak corresponding to [^{14}C]paf standard. Three more molecules possessing paf-related structures were tested next. Among them C18:0 lyso paf, the inactive precursor/metabolite of paf showed to be very active. C16:0 lyso paf and lyso phosphatidylcholine were less potent (Fig. 2) as well as the carbamyl analogue of paf (data not shown). The 2-acyl (long chain) analogue of paf, hexadecyl-palmitoyl-GPC (10 and 100 μM) was ineffective. This result led us to reconsider the specificity of

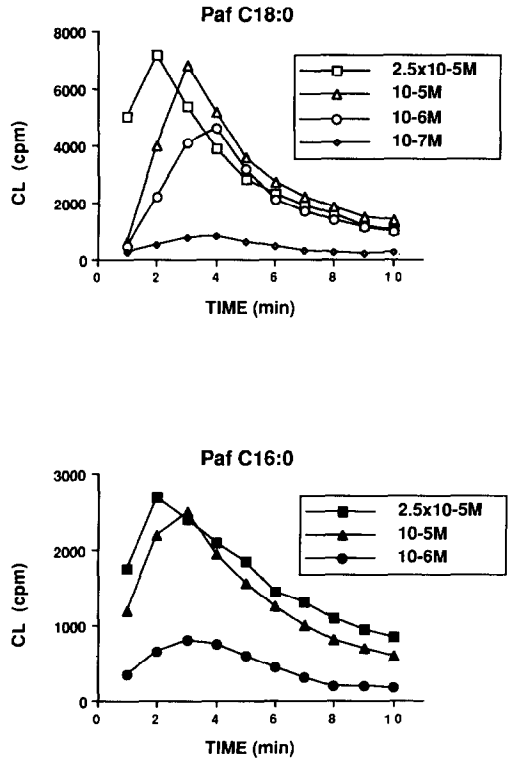

Fig. 1. Time-course and dose effects of paf C18:0 and C16:0-induced chemiluminescence on EBV-BLCL. The results are representative of 4 independent experiments.

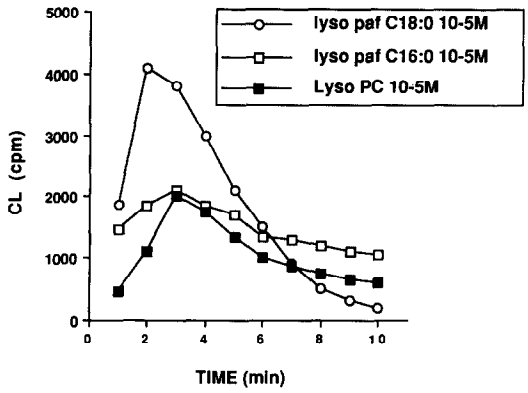

Fig. 2. Time-course of lyso paf C18:0, C16:0 and lyso PC-induced chemiluminescence on EBV-BLCL. The results are representative of 3 independent experiments.

paf action on EBV-BLCL. We thus tested three specific paf antagonists in order to counteract the paf-induced O_2^- formation by cells (Table I). Two of them, BN 52021 and WEB 2086, possessing structures non-related to paf, were inactive. In contrast the paf structural analogue CV 3988 inhibited the superoxide formation. The latter possessed also an early but weak agonist effect. In the above experiments all phospholipids tested were slightly toxic to cells since the cell viability (determined by the Trypan blue dye exclusion test) decreased from 95% to 80% after a 10 min incubation with phospholipids.

Taken together our data fit with detergent-like action of paf on EBV-BLCL rather than with a receptor-mediated process. First, the potency of paf with respect

Table I

Effect of paf inhibitors (BN, WEB and CV) on paf and lyso paf stimulated CL in EBV-B cells

Inhibitors	None	paf C18:0	paf C16:0	Lyso-paf C18:0
None	236 ± 40	6200 ± 656	2360 ± 525	3450 ± 150
BN 52021				
10-3M	246 ± 25	5483 ± 382	2116 ± 275	3100 ± 264
10-4M	183 ± 35	5667 ± 252	NT	NT
WEB 2086				
10-4M	300 ± 50	5250 ± 229	1916 ± 202	3083 ± 252
10-5M	180 ± 30	6030 ± 305	NT	NT
CV 3988				
10-3M	1570 ± 202[a]	933 ± 104	746 ± 126	990 ± 185
10-4M	873 ± 136[a]	4133 ± 351	NT	NT

[a] Effect of CV alone after 1 min incubation with B cells (the CL decreased to 700 cpm and 350 cpm after 3 min for 10^{-3} M and 10^{-4} M, respectively)

10^6 EBV-BLBL were stimulated with 10^{-5} M paf C18:0, C16:0 and lyso paf C18:0 in the presence and absence of 1 min preincubation of the different inhibitors. CL (in cpm) was measured at 3 min corresponding to the peak of the response. The controls contained buffer with or without inhibitors. Results represented mean ± SD ($n = 3$)

to O_2^- formation is very similar to that of lyso paf confirming former findings that the substitution of a hydroxyl group by a methyl, acetyl or benzyl group are without major effects on the hemolytic activity of such molecules [14]. In the majority of cellular or tissue models lyso paf is inactive in the non-lytic concentration range [2]. However, paf and lyso paf at micromolar concentrations, close to the critical micellar concentration are membrane perturbants [15]. Second, the superoxide anion formation by paf-challenged EBV-BLCL was not inhibited by both specific paf antagonists BN 52021 and WEB 2086. The inhibition of O_2^- release by CV 3988 might be attributed to the incorporation of this molecule into the cell membrane leading to a protection of the latter against paf attack. Third, several authors reported that the biological activity of C16:0 paf was 2–3 times superior to that of C18:0 paf (reviewed in [16]). In our hands the same paf dilutions tested for platelet aggregating activity revealed that C16:0 paf was 2- to 3-fold more potent than its C18:0 analogue. In contrast, when using the superoxide formation test on EBV-BLCL, C18:0 paf was about 3-fold more active than its C16:0 analogue.

Is the phospholipid-initiated superoxide production by B cells physiologically meaningful? In the inflammatory site paf and lyso paf concentration might be high since the stimulated polymorphonuclear neutrophils and monocyte/macrophages are a huge source of paf [2].

The small alterations induced in the cell membrane phospholipid (short chain) content may give rise to subtle changes of cellular surface appearance. This, together with the effects of these detergents on membrane-bound enzymes [15], would indicate a role of these molecules in membrane properties involved in the surface recognition process. Clinical trials using paf analogues that resist normal phospholipid metabolism, based on the idea of controlling cellular surface properties by pharmacological means are presently performed [17]. The present work shows that such pharmacological manipulations may elicit unexpected functional patterns among target cells.

Acknowledgements: We wish to thank Dr J. Benveniste for helpful discussion and F. Mitenne and G. Bessou for excellent technical assistance.

REFERENCES

[1] Travers, J.B., Li, Q., Kniss, D.A. and Fertel, R.H. (1989) J. Immunol. 143, 3708–3713.
[2] Benveniste, J. (1988) in: Biological Membranes: Aberrations in Membrane Structure and Function (Karnovsky, M.L., Leaf, A. and Bolis, L.C. eds) Liss, New York, pp. 73–85.
[3] Mauco, G., Chap, H. and Douste-Blazy, L. (1983) FEBS Lett. 153, 361–365.
[4] Shen, T.Y., Hwang, S.-B., Chang, M.N., Doebber, T.W., Lam, M.-H.T., Wu, M.S., Wang, X., Han, G.Q. and Li, R.Z. (1985) Proc. Natl. Acad. Sci. USA 82, 672–676.
[5] Nunez, D., Chignard, M., Korth, R., Le Couedic, J.-P., Norel, X., Spinnewyn, B., Braquet, P. and Benveniste, J. (1986) Eur. J. Pharmacol. 123, 197–205.
[6] Casals-Stenzel, J., Muacevic, G. and Weber, K.H. (1987) J. Pharmacol. Exp. Ther. 241, 974–981.
[7] Terashita, Z.I., Tsushima, S., Yoshioka, Y., Nomura, H., Inada, Y. and Nishikawa, K. (1983) Life Sci. 32, 1975–1981.
[8] Maly, F.E., Cross, A.R., Jones, O.T.G., Wolf-Vorbeck, G., Walker, C., Dahinden, C.A. and De Weck, A.L. (1988) J. Immunol. 140, 2334–2339.
[9] O'Flaherty, J.T., Wykle, R.L., Miller, C.H., Lewis, J.C., Waite, M., Bass, A., McCall, C.E. and DeChalet, L.R. (1981) Am. J. Pathol. 103, 70–78.
[10] Poitevin, B., Roubin, R. and Benveniste, J. (1984) Immunopharm. 7, 135–144.
[11] Vivier, E., Denizot, Y., Benveniste, J. and Thomas, Y. (1989) in: Cell Activation and Signal Initiation: Receptor and Phospholipase Control of Inositol Phosphate, PAF, and Eicosanoid Production, vol. 106 (Dennis, E.A., Hunter, T. and Berridge, M. eds) Liss, New York, pp. 337–344.
[12] Dugas, B., Vazques, A., Klein, B., Delfraissy, J.-F., Rammou, M., Gerard, J.-P. and Galanaud, P. (1986) Eur. J. Immunol. 16, 1609–1614.
[13] Tencé, M., Polonsky, J., Le Couedic, J.-P. and Benveniste, J. (1980) Biochimie 62, 251–259.
[14] Weltzien, H.U. (1975) Exp. Cell Res. 92, 111–121.
[15] Sawyer, D.B. and Andersen, O.S. (1989) Biochim. Biophys. Acta 987, 129–132.
[16] Pinckard, R.N., Ludwig, J.C. and McManus, L. (1988) in: Inflammation: Principles and Clinical Correlates (Gallin, J.I., Goldstein, I.M. and Snyderman, R. eds) Raven, New York, pp. 139–167.
[17] Berdel, W.E. and Munder, P.G. (1987) in: Platelet-Activating Factor and Related Lipid Mediators (Snyder, F. ed.) Plenum, pp. 449–467.