Biochemistry (Moscow), Vol. 67, No. 11, 2002, pp. 1248-1252. Translated from Biokhimiya, Vol. 67, No. 11, 2002, pp. 1511-1517.

Influence of Acyl and Plasmalogenic Analogs of Platelet Activating Factor on Chemotaxis of Human Leukocytes *in vitro* and Their Inflammatory and Antiinflammatory Activity *in vivo*

V. I. Kulikov and G. I. Muzya*

Research and Technology Center of Medical Biotechnology, Russian Federation Ministry of Public Health, ul. Shchukinskaya 6, Moscow, 123182 Russia; fax: (095) 190-0100

Received October 9, 2001

Abstract—The effects of platelet activating factor (PAF) and its cell analogs 1-O-alk-1′-enyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-alkenyl-PAF) and 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-acyl-PAF) on chemotaxis of human leukocytes *in vitro* and their inflammatory and antiinflammatory activities *in vivo* were studied. Both analogs stimulated chemotaxis of human leukocytes in agarose gel. PAF and 1-alkenyl-PAF induced rat paw edema in the range of doses 0.1-10 and 10-100 μg per paw, respectively. Paw edema induced by 1-acyl-PAF (10-100 μg per paw) was more pronounced than that induced by PAF or 1-alkenyl-PAF. The latter also exhibited significant antiinflammatory effect by inhibiting PAF- or carrageenan-induced rat paw edema, and this effect exceeded that of dexamethasone. In these models of inflammation 1-acyl-PAF did not exhibit any antiinflammatory activity. The data suggest that PAF is not the only cell phospholipid mediating inflammation—its cell analogs, 1-acyl-PAF and 1-alkenyl-PAF, may also be involved into the inflammatory response. Possible interrelationships between cellular synthesis of 1-acyl-PAF, its formation in oxidized LDL, biological effects of lysolecithin, and penetration of LDL into the arterial wall are discussed.

Key words: platelet activating factor (PAF), PAF cell analogs, chemotaxis, leukocytes, inflammation

Subcutaneous administration of platelet activating factor (PAF; 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) to man or animals is accompanied by edema, endothelial barrier dysfunction, thrombus formation, and release of other cellular mediators such as interleukins, leukotrienes, prostaglandins, and tumor necrosis factor (TNF). This suggests a key role of PAF as an inflammatory mediator [1]. Some cytokines (interleukin-1, -2, -6, -8, interferon, TNF) and growth factors (platelet-derived growth factor (PDGF) and granulocyte-monocyte colony stimulating factor (GM-CSF)) related to PAF are known to be involved in inflammatory processes of various etiology and in atherogenesis [2].

Penetration of lipoproteins into the subendothelial space (intima) is one of the early stages of atherosclerosis [2]. Low density lipoproteins (LDL) bind to endothelial cells at apoB- and apoE-receptors, whereas modified lipoproteins interact with scavenger-receptors [2]. Some plasma lipoproteins are believed to penetrate into arterial intima in endothelial cell-independent manner via intercellular spaces, which are normally very narrow (3-4 nm) and impermeable for high molecular weight compounds. Vasoactive regulators (adrenalin, noradrenalin, serotonin, angiotensin II, bradykinin) released into the blood stream or high blood cholesterol content enlarge the intercellular distance up to 10-20 nm, and this is sufficient for LDL penetration into arterial intima [2]. Such events may also take place in local and generalized inflammation. PAF and TNF are known to increase microvascular permeability by modulating NO synthesis [3]; they also stimulate neutrophil and monocyte adhesion to endothelium [4] and release of biologically active substances promoting both inflammatory reaction and activation of cells involved in atherogenesis. Monocytes and foam cells may synthesize PAF [5], whereas acyl analog of PAF, 1-acyl-PAF, was found in oxidized LDL [6].

In blood plasma PAF is mainly associated with LDL and (partially) with high density lipoproteins (HDL) [7].

Abbreviations: PAF) platelet activating factor (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); 1-alkenyl-PAF) 1-O-alk-1′-enyl-2-acetyl-*sn*-glycero-3-phosphocholine; 1-acyl-PAF) 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine; HDL) high density lipoproteins; LDL) low density lipoproteins.

* To whom correspondence should be addressed.

0006-2979/02/6711-1248$27.00 ©2002 MAIK "Nauka/Interperiodica"

Addition of exogenous PAF (0.1-10 nM) to human plasma inhibited cholesterol etherification catalyzed by lecithin cholesterol acyltransferase [8]. We recently demonstrated that acyl and plasmalogenic PAF analogs, 1-acyl-PAF and 1-alkenyl-PAF, respectively, stimulated superoxide radical formation in isolated blood leukocytes of healthy donors, whereas hyperproduction of superoxide radicals in leukocytes from patients with hypercholesterolemia was a PAF- or PAF-like lipid-dependent process [9]. Systemic effects induced by PAF analogs (1-acyl-PAF and 1-alkenyl-PAF) are still poorly characterized. Certain evidence exists (e.g., hypotensive and cardiodepressor effects) that their biological activity differs from that of PAF [10]. Better understanding of the interrelationship between inflammatory and processes requires comparative analysis of inflammatory activity of PAF and its cell analogs.

In the present study we investigated effects of 1-acyl-PAF and 1-alkenyl-PAF on chemotaxis of human leukocytes *in vitro* and their inflammatory and antiinflammatory activities *in vivo*.

MATERIALS AND METHODS

The following chemicals were used in the study: dextran T-500, Ficoll-Paque reagent (Pharmacia, Sweden); acetylsalicylic acid, carrageenan (type IV) (Sigma, USA); dexamethasone (Cadila Laboratories, India); Hanks medium (Virion, Tomsk, Russia).

PAF was obtained from bovine heart choline plasmalogens as described earlier [11]. 1-Acyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-acyl-PAF) was prepared by acetylation of 1-acyllyso-*sn*-glycero-3-phosphocholine with acetic anhydride and purified by column chromatography on silica gel [12]. 1-O-Alk-1′-enyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-alkenyl-PAF) was prepared by acetylation of 1-O-alk-1′-enyl-2-acetyl-*sn*-glycero-3-phosphocholine with acetic anhydride, purified, and characterized as described [13].

Leukocyte enriched cell suspension was isolated from fresh human donor blood by erythrocyte sedimentation with dextran T-500 followed by subsequent centrifugation in a single step Ficoll-Paque gradient [14].

The effect of PAF and its cell analogs on leukocyte chemotaxis was investigated in agarose gel according to the method of Beznosenko et al. [15]. Briefly, central and peripheral wells in the agarose gel were filled with leukocyte suspension (10 μl, 250,000 cells) and the analyzed compound, respectively. The agarose gel dishes were incubated at 37°C for 3 h, then fixed with 10% formalin and 2 h later evaluated leukocyte chemotaxis by using microscope at 70-fold magnification. The chemotaxis index (CI) was used for quantitative evaluation. It was calculated as follows: CI = A/B, where A and B are the distances covered by cells towards the medium with and without chemoattractant, respectively.

The inflammatory activity of PAF and its analogs was evaluated by paw edema, which was induced in Wistar rats (180-220 g) by subplantar injection of 100 μl of PAF or its analogs. Their doses varied from 0.1 to 100 μg per paw. The volume of paws was measured by an automated liquid plethysmometer before (0 min) and 60 min after injection. The change in the paw volume was calculated as the difference in paw volumes at 0 and 60 min. Control animals received subplantar injection of Hanks medium of the same volume (100 μl).

For evaluation of the antiinflammatory activity, PAF and its analogs were injected intraperitoneally in the range of doses 0.001 to 10 mg per kg body weight (in 0.5 ml) 60 min before the subplantar injection of PAF (2.5 μg per paw) or carrageenan (750 μg per paw).

The results were treated by using unpaired statistical criteria and Student's *t*-test. Data represent mean ± SEM.

RESULTS AND DISCUSSION

Chemotactic factors are known to induce directed migration of leukocytes [15]. PAF, one of the inflammatory mediators, can stimulate chemotaxis of isolated leukocytes *in vitro* [1]. Table 1 shows effects of PAF, 1-acyl-PAF, and 1-alkenyl-PAF on chemotaxis of human blood leukocytes. In

Table 1. Effect of PAF, 1-alkenyl-PAF, and 1-acyl-PAF at various concentrations on chemotaxis of isolated human blood leukocytes

Compound/concentration	Chemotactic leukocyte index			
	0.001 μM	0.1 μM	10 μM	100 μM
PAF	1.1 ± 0.02	1.12 ± 0.04	1.2 ± 0.05	1.3 ± 0.07
1-Alkenyl-PAF	1.13 ± 0.12	1.13 ± 0.05	1.24 ± 0.04	1.23 ± 0.09
1-Acyl-PAF	1.1 ± 0.04	1.14 ± 0.08	1.17 ± 0.14	1.30 ± 0.14

the range of concentrations 0.001-10 μM these substances moderately stimulated leukocyte chemotaxis. Increase of their concentration to 100 μM resulted in the increase of PAF- and 1-acyl-PAF-induced leukocyte chemotaxis, whereas 1-alkenyl-PAF did not cause further stimulation of this parameter. Since both PAF analogs stimulate leukocyte chemotaxis it is possible that they may be involved into the development of inflammatory reaction. So we have investigated the effect of subplantar administration of 1-acyl-PAF and 1-alkenyl-PAF on rat paw edema.

Table 2 shows the dependence of inflammatory reaction on the dose of PAF, 1-acyl-PAF, and 1-alkenyl-PAF. In the case of PAF and 1-alkenyl-PAF the development of paw edema was characterized by a clear maximum, which was observed at the dose of PAF and 1-alkenyl-PAF of 2.5 and 10 μg per paw, respectively. (Since maximal paw edema after a single injection of PAF (2.5 μg per paw) developed to 60 min (data not shown), this time interval was used in subsequent experiments.) In contrast to PAF and 1-alkenyl-PAF, the dependence of paw edema on the dose 1-acyl-PAF was characterized by a linear increase over the whole range of doses up to 100 μg per paw. At this dose of 1-acyl-PAF maximal inflammatory reaction (1050 ± 68.7 μl) was two times higher than that observed at the same dose of PAF.

At very low concentrations (0.01-1 nM) PAF can cause desensitization of target cells to subsequent effect of higher concentrations of this compound. This effect was also observed in the case of PAF-induced inflammation [16]. So we investigated the possible influence of PAF cell analogs on PAF-induced inflammation. Table 3 shows antiinflammatory activity of PAF, 1-alkenyl-PAF, and 1-acyl-PAF. Intraperitoneal administration of PAF (0.005-0.1 mg per kg) to rats inhibited the development of paw edema induced by subplantar administration of PAF. In the range of doses 0.01-1.0 mg/kg 1-alkenyl-PAF inhibited paw edema more effectively than PAF. It should be mentioned that systemic administration of PAF is toxic and its dose 1.0 mg/kg is lethal for rats (LD_{100}), whereas 1-alkenyl-PAF is not toxic at all at least up to dose 10 mg/kg. In contrast to PAF and 1-alkenyl-PAF, 1-acyl-PAF did not demonstrate any antiinflammatory activity in the range of doses 0.1-10 mg/kg. Administration of standard antiinflammatory compounds aspirin and dexamethasone in routine doses also inhibited PAF-induced paw edema (Table 3). At the dose 1 mg/kg 1-alkenyl-PAF

Table 2. Inflammatory activity of PAF, 1-alkenyl-PAF, and 1-acyl-PAF

Compound	Dose, μg per paw	Change of paw volume, μl
Control (Hanks medium, 100 μl)	—	32 ± 0.9
PAF	0.1	217 ± 70.9
	0.5	282 ± 38.5
	1.0	384 ± 65.3
	2.5	592 ± 49.4
	5.0	506 ± 36.5
	10.0	360 ± 69.2
1-Alkenyl-PAF	1.0	285 ± 43.3
	5.0	470 ± 28.2
	10.0	783 ± 38.4
	25.0	720 ± 54.9
	100.0	365 ± 66.3
1-Acyl-PAF	1.0	128 ± 24.2
	10.0	233 ± 37.5
	25.0	504 ± 60.5
	50.0	730 ± 50.3
	100.0	1050 ± 68.7

Note: In all cases $p < 0.05$ compared with control.

Table 3. Antiinflammatory activity of PAF, 1-alkenyl-PAF, and 1-acyl-PAF in the model of PAF-induced paw edema

Compound tested	Dose, mg per kg weight	Change of paw volume, % of control
Control (Hanks medium, 0.5 ml)	—	100 ± 5.6
PAF	0.001	95 ± 19.8*
	0.005	58 ± 19.0
	0.01	55 ± 7.9
	0.1	34 ± 6.5
	1.0	LD100
1-Alkenyl-PAF	0.01	69 ± 2.5
	0.1	49 ± 7.1
	1.0	12 ± 9.4
1-Acyl-PAF	1.0	88 ± 42.0*
	10.0	124 ± 35.0*
Acetylsalicylic acid	200.0	76 ± 5.9*
Dexamethasone	0.5	65 ± 18.1
	1.0	43 ± 11.5

* $p > 0.05$.

Table 4. Influence of 1-alkenyl-PAF and the antiinflammatory drugs on the time-course of carrageenan-induced paw edema

Compound	Dose, mg per kg weight	Change of paw volume, % of control			
		1 h	3 h	5 h	24 h
Control (Hanks medium, 0.5 ml)	–	100 ± 26.4	100 ± 17.5	100 ± 18.4	100 ± 24.0
1-Alkenyl-PAF	0.1	68 ± 35.8*	36 ± 8.0	31 ± 9.9	9 ± 21.4
Acetylsalicylic acid	200	58 ± 29.5*	48 ± 9.4	77 ± 8.7*	110 ± 18.8*
Dexamethasone	1.0	87 ± 30.4*	25 ± 3.7	77 ± 15.2*	65 ± 28.6*

* $p > 0.05$.

was more effective in the inhibition of PAF-induced paw edema than dexamethasone. High antiinflammatory activity of 1-alkenyl-PAF is interesting not only in terms of its interaction with PAF but also for analysis of possible involvement of PAF in inflammatory processes of other etiology. Table 4 shows antiinflammatory activity of 1-alkenyl-PAF in the case of paw edema induced by subplantar administration of carrageenan, a mixture of polysaccharides from red marine algae. At the dose 0.1 mg/kg 1-alkenyl-PAF effectively inhibited carrageenan-induced paw edema, and this effect was more pronounced than the antiinflammatory effect of dexamethasone used in much higher dose, 1 mg/kg. In collaboration with scientists from P. Fabrez Drug Center (France) we demonstrated that administration of 1-alkenyl-PAF (0.1 mg/kg) inhibited lung edema induced by intrapleural PAF administration (data not shown).

The data suggest high inflammatory activity of 1-acyl- and 1-alkenyl-PAF; the latter also exhibits high antiinflammatory activity. Comparative analysis of inflammatory activity of PAF and its analogs revealed significant differences in doses producing the paw edema. PAF induced paw edema in the range of doses 1-5 μg per paw. In accordance with literature data administration of higher doses resulted in reduced inflammatory reaction [16]. 1-Alkenyl-PAF-induced dose response inflammatory reaction was similar to that of PAF; it was mainly registered in the range of doses from 5 to 25 μg per paw. In the case of 1-acyl-PAF the inflammatory reaction was observed at the dose 10 μg per paw and administration of a higher dose (up to 100 μg per paw) caused further increase in the inflammatory reaction. Thus, considering dose response range and manifestation of the inflammatory activity the efficacy of compounds studied decreased in the following order: 1-acyl-PAF > 1-alkenyl-PAF > PAF. Interestingly, in most cells and tissues the content of precursors for biosynthesis of these phospholipids (1,2-diacyl-*sn*-glycero-3-phosphocholines, 1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholines, 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine) decreased in the same order. Interpreting possible biological importance of these results we may suggest that among these cellular phospholipid metabolites PAF is responsible for the primary effect (inflammatory activity), which is observed at very low concentrations. Intensification of pathological processes in tissues is accompanied by activation of phospholipases A_2 hydrolyzing diacyl- and plasmalogenic phosphoglycerides. This results in formation of significant amounts of lyso-derivatives of these phosphoglycerides which can serve as substrates for synthesis of 1-acyl-PAF and 1-alkenyl-PAF catalyzed by acetyl transferase. These newly synthesized analogs of PAF may contribute to the development of inflammatory and other pathological processes. Data on high levels of 1-acyllyso-*sn*-glycero-3-phosphocholines (lysolecithins) seen in blood during myocardial ischemia [17], atherosclerosis [18], or asthma [19] seem to support this hypothesis.

Discovery of high antiinflammatory activity of 1-alkenyl-PAF in two models of inflammation (subplantar injection of PAF and carrageenan) is another important result of the present study. It is possible that systemic administration of 1-alkenyl-PAF causes desensitization of surface PAF-receptors on cells involved into the development of inflammatory processes. Such desensitization was demonstrated in the case of human and rabbit platelets [20]. We already suggested that 1-alkenyl-PAF may have some protective function acting as natural cellular PAF antagonist in the human cardiovascular system [13]. Data of the present study on high antiinflammatory activity of 1-alkenyl-PAF support our hypothesis.

High inflammatory activity of 1-acyl-PAF may be related to pathological processes in the cardiovascular system especially if we take into consideration some data on 1-acyllyso-*sn*-glycero-3-phosphocholine, known as lysolecithin. Accumulation of lysolecithin was found in ischemic myocardium [17]. Lysolecithin influences the tune of cardiac contractions [21], increases coronary blood flow, and reduces vascular resistance and blood

pressure [22]. Lysolecithin also induces monocyte adhesion to endothelial cells [23], increases expression of P-selectin in endothelial cells [24], and inhibits endothelial-dependent relaxation of rabbit aorta and pig mesenteric artery [25]. Since all these tissue contain acyl transferase catalyzing transfer of acetyl group from acetyl-CoA to lysolecithin with formation of 1-acyl-PAF numerous biological effects of lysolecithin may be (at least partially) attributed its conversion into 1-acyl-PAF. Such suggestion is quite possible because lysolecithin can penetrate inside cells containing this enzyme. The main mechanism of cell stimulation by lysolecithin involves protein kinase C activation [26]. However, it is also known that in some cells *de novo* synthesized 1-acyl-PAF is an intracellular regulator [20]. So, it is possible that protein kinase C is one of its targets.

High inflammatory activity of 1-acyl-PAF, biosynthesis of 1-acyl-PAF in various cells, and formation of 1-acyl-PAF in LDL [6] suggest possible existence of an interrelationship between 1-acyl-PAF, PAF and LDL penetration into arterial wall, which may be attributed to the increase of vascular permeability and the effects of PAF and 1-acyl-PAF on endothelial cells, neutrophils, monocytes, and macrophages.

Data of the present report also suggest that PAF is not the only cell phospholipid that can induce inflammation; 1-alkenyl-PAF and 1-acyl-PAF may be involved in the development of inflammatory processes as well.

REFERENCES

1. Braquet, P., Touqui, L., Shen, T. Y., and Vargaftig, D. B. (1987) *Pharmacol. Rev.*, **39**, 97-145.
2. Klimov, A. N., and Nikulcheva, N. G. (1999) *Lipid and Lipoprotein Metabolism and Its Impairments* [in Russian], Piter Com, St. Petersburg.
3. Ramirez, M. M., Quardt, S. M., Kim, D., Oshiro, H., Minnicozzi, M., and Duran, W. N. (1995) *Microvasc. Res.*, **50**, 223-234.
4. Smalley, D. M., Childs, E. W., and Cheung, L. Y. (2000) *Inflammation*, **24**, 399-410.
5. Dentan, C., Lesnik, P., Chapman, M. J., and Ninio, E. (1996) *Eur. J. Biochem.*, **236**, 48-55.
6. Tokumura, A., Toujima, M., Yoshioka, Y., and Fukuzawa, K. J. (1996) *Lipids*, **31**, 1251-1258.
7. Muzya, G. I., and Kulikov, V. I. (1991) *Biokhimiya*, **56**, 1140-1144.
8. Bergelson, L. D., Kulikov, V. I., and Muzya, G. I. (1985) *FEBS Lett.*, **190**, 305-306.
9. Kulikov, V. I., and Muzya, G. I. (2002) *Biochemistry (Moscow)*, **67**, 662-666.
10. Kulikov, V. I., and Muzya, G. I. (1998) *Biochemistry (Moscow)*, **63**, 47-54.
11. Demopoulos, C. A., Pinckard, R. N., and Hanahan, D. J. (1979) *J. Biol. Chem.*, **254**, 9355-9358.
12. Orlov, S. A., Kulikov, V. I., Polner, A. A., and Bergelson, L. D. (1985) *Biokhimiya*, **50**, 680-685.
13. Kulikov, V. I., and Muzya, G. I. (1999) *Biochemistry (Moscow)*, **64**, 6331-635.
14. Orlov, S. A., and Kulikov, V. I. (1987) *Immunologiya*, **3**, 33-35.
15. Beznosenko, S. A., Barsukov, A. A., and Zemskov, V. M. (1984) *Zh. Mikrobiol.*, **6**, 101-106.
16. Bonnet, J., Loiseau, A. M., Orvoen, M., and Bessin, P. (1981) *Agents Action*, **11**, 559-562.
17. Kinnaird, A. A. A., Choy, P. C., and Man, R. Y. K. (1988) *Lipids*, **23**, 32-35.
18. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. M. (1989) *New Engl. J. Med.*, **320**, 915-924.
19. Mehta, D., Gupta, S., Gaur, S. N., Gangal, S. V., and Agrawal, K. P. (1990) *Am. Rev. Respir. Dis.*, **142**, 157-161.
20. Kulikov, V. I., and Muzya, G. I. (1996) *Biochemistry (Moscow)*, **61**, 289-298.
21. Giffin, M., Arthur, G., Choy, P. C., and Man, R. Y. K. (1988) *Can. J. Physiol. Pharmacol.*, **66**, 185-189.
22. Wolf, A., Saito, T., Dudek, R., and Bing, R. J. (1991) *Lipids*, **26**, 223-226.
23. Nakano, T., Raines, E. W., Abraham, J. A., Klagsburn, M., and Ross, R. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 1069-1073.
24. Sugiyama, S., Kugiyama, K., Ohgushi, M., Fujimoto, K., and Yasue, H. (1994) *Circ. Res.*, **74**, 565-575.
25. Fukao, M., Hattori, Y., Kanno, M., Sakuma, I., and Kitabatake, A. (1995) *Br. J. Pharmacol.*, **116**, 1541-1544.
26. Oishi, K., Raynor, R. L., Charp, P. A., and Kuo, J. F. (1988) *J. Biol. Chem.*, **263**, 6865-6871.