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Platelet activating factor (PAF) effects on ciliary activity of human paranasal sinus mucosa in vitro

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SUMMARY

The effect of PAF on ciliary activity was investigated in vitro. Normal human paranasal sinus mucosa was obtained from the ethmoid sinuses by surgical procedure and incubated in the form of tissue culture. Mucosal surface profile was viewed under an inverted microscope and ciliary activity was photoelectrically measured. Ciliary inhibition was significantly induced after a 60 min period of incubation with 10^{-8} M PAF in vitro followed by irrigation. However, when the mucosa was irrigated after a 15 min incubation period the ciliary activity showed no remarkable change. The effect of 10^{-8} M PAF on ciliary activity was completely blocked when pre-incubated and then incubated with 10^{-6} M CV-3988 (a specific PAF receptor antagonist); however, it was moderately inhibited when only preincubated with CV-3988. These data indicate that PAF specifically affects ciliated cells in the first 60 min after the challenge.

INTRODUCTION

There are many chemical mediators reported to be involved in allergic and inflammatory disorders. In patients with nasal allergy and bronchial asthma, their respiratory tract is hyperresponsive to non-specific stimuli such as histamine (Cockcroft et al., 1983; Okuda et al., 1983). Characteristically, it is hypothesized that this phenomenon is a result from the damage of the mucosal epithelium, which may be induced by free radical (Pincus et al., 1982) and inflammatory cells, particularly eosinophils (Barnes et al., 1986). PAF is predominantly synthesized from eosinophils (Lee et al., 1984) rather than neutrophils (Lotner

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et al., 1980), platelets (Chignard et al., 1979), macrophages (Arnoux et al., 1980) and mast cells (Schleimer et al., 1986). Recently many researchers have taken a great interest in the cytotoxicity of mediators released from eosinophils, such as MBP: Major Basic Protein (Frigas et al., 1980; Hisamatsu et al., 1990), ECP: Eosinophil Cationic Protein, and EPO: Eosinophil Peroxidase (Motojima et al., 1989). We reported PAF inhibited ciliary activity of human paranasal sinus mucosa (Ganbo et al., 1990). In this paper we describe the specific effect of PAF on ciliary activity of human paranasal sinus mucosa (in vitro) in an experiment conducted by washing the mucosal specimens after exposure to PAF and then incubating it with a specific PAF antagonist, CV-3988.

MATERIALS AND METHODS

Maintenance of the human paranasal sinus mucosa

Normal paranasal sinus mucosa with ciliated epithelial cells was removed by surgical procedure from the ethmoid sinuses of patients who had suffered facial trauma. The mucosa was rinsed in Eagle's MEM to remove blood cells and mucus, and incubated for more than 48 hrs in the manner of tissue culture. The culture medium consisted of Eagle's MEM with 5% FCS. Only non-infiltrated mucosa without inflammatory cells was obtained.

PAF and its antagonist

PAF: 1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine (Bachem Feinchemikalien AG, Bubendorf, Switzerland) and CV-3988: (RS)-2-Methoxy-3-(octadecylcarbamoyloxy)propyl 2-(3-thiazolio)ethyl phosphate (Takeda Chemical Industries Ltd, Osaka, Japan), which is a highly specific PAF inhibitor, were used. PAF was dissolved in methanol at 10^{-2} M and then further diluted with Eagle's MEM to 10^{-8} M. CV-3988 was dissolved in 50 °C physiological saline at 10^{-3} M and then diluted with the medium to 10^{-6} M.

Observation and recording of ciliary activity and mucosal surface profile

Ciliary activity was viewed at 37 °C under an inverted microscope equipped with a thermoregulator and a humidified CO_2 chamber, recorded on the video tapes and photoelectrically measured. Ten ciliated cells were observed in each experiment and their values were expressed as mean ± SD. If the number of ciliostatic cells reached more than 25% of all observed cells, the observation of ciliary activity was discontinued. The alteration of the surface profile was also observed in the same manner. The exfoliation of observed ciliated cells was assessed according to the following criteria: Grade 0 = smooth profile and no exfoliated cells, Grade 1 = less than 25% change, Grade 2 = 25% to 50%, Grade 3 = 50% to 75%, Grade 4 = 75% to 100%. The ciliostatic alteration of ciliated cells was also assessed using the same grading.

Statistics

The significant difference between recorded values was statistically determined at $p<0.01$ on the Student's t-test.

RESULTS

The paranasal sinus mucosa was incubated with 10^{-8} M PAF and its ciliary activity was time-dependently inhibited (the control group). When the mucosa was irrigated three times with Eagle's MEM after a 15 min exposure period to 10^{-8} M PAF and incubated with Eagle's MEM (I-A), the time course of ciliary activity showed no remarkable change. However, when irrigated after a 60 min exposure period to 10^{-8} M PAF (I-B), the time course of ciliary activity was the same as 10^{-8} M PAF with no irrigation (the control). The significant differences between non-irrigation (the control) and irrigation after a 15 min exposure (I-A); and a 15 min (I-A) and a 60 min exposure (I-B) are shown after 2 hrs and 4 hrs ($p<0.01$) in Figure 1. When the mucosa was incubated with 10^{-8} M PAF after a 15 min preincubation with 10^{-6} M CV-3988, ciliary activity was moderately inhibited by PAF (II-A). When the mucosa was incubated with 10^{-6} M CV-3988 and 10^{-8} M PAF after a 15 min preincubation with 10^{-6} M CV-3988, ciliary activity showed no remarkable change (II-B). The significant differences of values between non-preincubation (the control) and preincubation with CV-3988 (II-A); and preincubation with CV-3988 (II-A) and preincubation and incubation with CV-3988 (II-B) are shown after 4 hrs ($p<0.01$) in Figure 2. Alterations of the mucosal surface profile are shown in Table 1. At a concentration of 10^{-8} M PAF, exfoliation and ciliostasis appeared after 6 and 8 hrs. Irrigating after a 15 min exposure to PAF, neither exfoliation nor ciliostasis appeared throughout the 24 hrs period. Irrigating after a 60 min exposure, exfoliation and ciliostasis appeared after 8 and 12 hrs respectively. The length of the time for alterations of the profile to appear was slightly of prolonged as compared with non-irrigation. Preincubation with CV-3988 followed by incubation with only PAF resulted in alterations appearing after a significantly prolonged period of time. Although ciliary activities were evidently inhibited, exfoliation and ciliostasis did not appear until after 20 hrs. Incubation with PAF and CV-3988 after preincubation with CV-3988, showed no remarkable alteration of the mucosal profile.

DISCUSSION

In this paper we employed 10^{-8} M PAF to study the mode of PAF action to ciliary activity of paranasal sinus mucosa in vitro in a follow-up to our previous experiments (Ganbo et al., 1990). The obtained results show that an exposure period of 60 min to PAF is enough time to inhibit ciliary activity. The time course of the ciliary activity (I-B) was the same as that without irrigation (the control) although some differences in the alteration of the mucosal surface profile were observed.

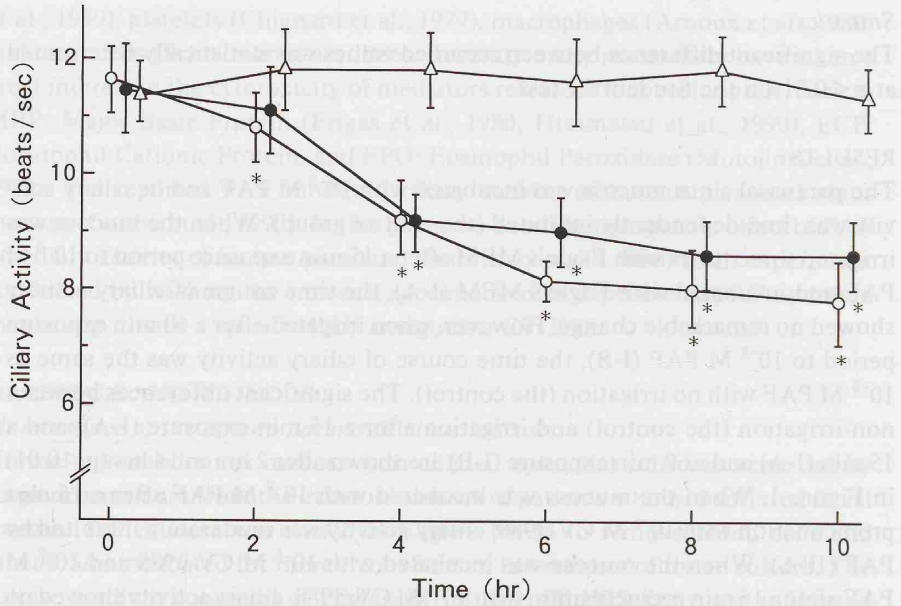

Figure 1. 10^{-8} M PAF with irrigation.
-O- (the control group): 10^{-8} M PAF incubation only,
-△- (I-A): irrigation after a 15 min exposure period to 10^{-8} M PAF,
-●- (I-B): irrigation after a 60 min exposure period to 10^{-8} M PAF,
*: $p<0.01$

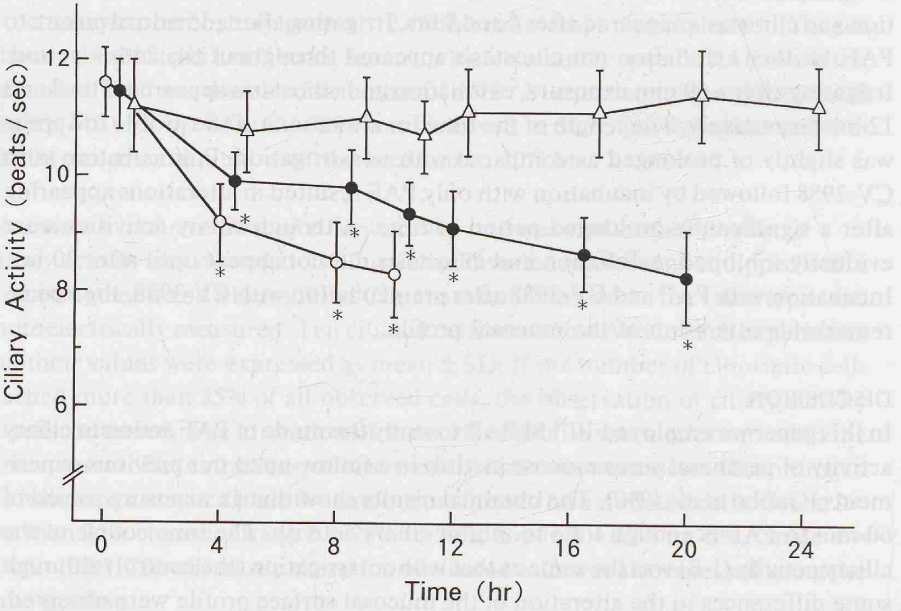

Figure 2. 10^{-8} M PAF with 10^{-6} M CV-3988.
-O- (the control): 10^{-8} M PAF incubation only,
-●- (II-A): preincubation with 10^{-6} M CV-3988 followed by 10^{-8} M PAF incubation,
-△- (II-B): preincubation with 10^{-6} M CV-3988 followed by incubation with both 10^{-6} M CV-3988 and 10^{-8} M PAF.
*: $p<0.01$

Table 1. Alterations of the mucosal surface profile.

mucosal surface profile	test solution		Exposure Time (hr) 0	2	4	6	8	10	12	14	16	18	20	22	24
exfoliation	10^{-8}M PAF		0	0	0	1	2	3	4						
	irrigation	after 15 min	0	0	0	0	0	0	0	0	0	0	0	0	0
		after 60 min	0	0	0	0	1	2	3	4	4	4	4		
	CV-3988	preincubation	0	0	0	0	0	0	0	0	0	0	0	1	2
		preincubation + incubation	0	0	0	0	0	0	0	0	0	0	0	0	0
ciliostasis	10^{-8}M PAF		0	0	0	0	1	1	4						
	irrigation	after 15 min	0	0	0	0	0	0	0	0	0	0	0	0	0
		after 60 min	0	0	0	0	0	0	1	2	3	4	4		
	CV-3988	preincubation	0	0	0	0	0	0	0	0	0	0	0	2	4
		preincubation + incubation	0	0	0	0	0	0	0	0	0	0	0	0	0

This could possibly be a result of the mucosa being affected by PAF primarily in the first 60 min after the challenge, and not throughout the remainder of the test period, since PAF might be rendered inactive across time by the metabolic enzyme or environmental agents such as temperature and light. PAF is changed to lyso-PAF (an inactive metabolite) by acetylhydrolase which exists in the cytosolic fraction of a variety of tissues (Blank et al., 1981). PAF might connect its receptor on the ciliated cells and changed to be lyso-PAF by acetylhydrolase generated in these cells. However, the medium which contained 10^{-8} M PAF was irrigated after a 15 min exposure of PAF (I-A) and no remarkable change in the time course of the ciliary activity and the mucosal surface profile was observed. The data suggest that in order for PAF to induce ciliary inhibition at a concentration of 10^{-8} M, an exposure period of more than 15 min is required.

CV-3988 was first reported as a specific inhibitor of PAF by Terashita et al. (1983). CV-3988 was used in our experiments because we found in a preliminary experiment that ciliary activity was not inhibited by CV-3988 following a 24 hr period of incubation. We employed a concentration of 10^{-6} M CV-3988 to 10^{-8} M PAF. Terashita et al. (1985) reported CV-3988 (1.6×10^{-5} M) inhibited aggregation of human platelet induced by PAF (10^{-7} to 10^{-6} M), so from this we can surmise that the concentration of CV- 3988 needs about one hundred times that of PAF in order to inhibit PAF's effect on ciliary activity. The effect of PAF on ciliary activity was completely blocked when the ciliated cells were preincubated and incubated with CV-3988 (II-B). We hypothesize that ciliary inhibition resulted by PAF being bound to its specific receptor. Presently the PAF receptor is not clarified well. However, the interaction of PAF with rabbit platelets is partially understood and could be divided into three different components:

1. the specific and reversible one which has high affinity,
2. the specific and irreversible one which has low affinity,
3. the non-specific and irreversible one.

Component 1 is considered to be the receptor associated with the biological response to PAF (Homma et al., 1987).

Ciliary activity was moderately inhibited by PAF when only preincubated with CV-3988 (II-A). The effect of PAF on ciliary activity was not completely blocked. Both preincubation and incubation with CV-3988 were required in order to completely inhibit the PAF induced ciliary dysfunction and mucosal damage. These data suggest that there may be a competition between PAF and CV-3988. The effect of PAF on ciliated cells is considered to be direct because the cultured mucosa was not infiltrated with inflammatory cells which indirectly releases other mediators when stimulated by PAF. These results indicate that ciliary inhibition was directly induced by the specific effect of PAF.

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