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Turn-over of PAF in cultures of human paranasal sinus mucosa*

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SUMMARY

The change of PAF concentration in the culture medium was investigated by radioimmunoassay when 10^{-8} M PAF or 10^{-8} M lyso-PAF was incubated with a piece of normal human paranasal sinus mucosa. The PAF concentration in the medium of the former group was halved within 11.3 min and reduced to less than 5% of the initial concentration within 60 min. However, there was no significant difference in the reduction of PAF concentrations in the medium between groups with or without the mucosa. When 10^{-8} M lyso-PAF was incubated with a piece of mucosa, PAF gradually increased and reached the maximum of 0.36×10^{-8} M at 20 min, and thereafter quickly decreased to a non-detectable level.

Key words: paranasal sinus mucosa, cell culture, platelet-activating factor

INTRODUCTION

Platelet-activating factor (PAF) not only activates platelets (Kloprogge et al., 1983), but also other inflammatory cells such as eosinophils (Wardlaw et al., 1986), neutrophils (Shaw et al., 1981), and monocytes (Yasaka et al., 1982). In addition, PAF has biological activities: contraction of smooth muscle (Findlay et al., 1981), increase of vascular permeability (Humphrey et al., 1984), and hypersecretion of tracheal submucosal glands (Hahn et al., 1985). We recently reported that PAF induces ciliary inhibition of human paranasal sinus mucosa *in vitro* (Ganbo et al., 1990). Ciliary inhibition was observed in a dose-dependent manner at concentrations ranging from 10^{-10} M to 10^{-6} M after several hours of incubation with PAF. However, it is not supposed that PAF can continuously affect ciliary activity of the mucosal epithelium for several hours. Because the biological activity of PAF might be unstable, PAF could be rapidly metabolized to an inactive form by tissue enzymes or environmental factors, such as temperature and light. It is not well known that PAF reduces activity over time when being incubated with paranasal sinus mucosa. To determine the time course of the variation in PAF concentration during culture of the mucosa, the concentrations in the culture medium containing PAF and lyso-PAF were observed over a period of time by radioimmunoassay.

MATERIAL AND METHODS

Maintenance of human paranasal sinus mucosa

Normal mucosa was surgically removed from the ethmoidal sinuses of patients suffering from facial bone fracture. We got informed consent for the use of mucosa from all patients. The removed mucosa was rinsed in Eagle's Minimal Essential Medium (MEM), and cut into pieces of approximately 4×4 mm with sharp scissors. The mucosal specimen were transferred into a chamber of 35×10 mm and incubated in Eagle's MEM containing 10% Fetal Calf Serum (FCS) for several days, to remove mucus and cellular debris.

PAF and lyso-PAF

PAF (1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) and lyso-PAF (1-0-hexadecyl-sn-glycero-3-phosphorylcholine) were used (Bachem Feinchemikalien AG; Bubendorf, Switzerland). PAF and lyso-PAF were dissolved in methanol at a concentration of 10^{-2} M and diluted with Eagle's MEM to 10^{-8} M. The mucosal specimen were incubated in 1.5 ml of each test solution.

Radioimmunoassay of PAF

The concentration of PAF in the culture medium was measured by a [^{125}I]PAF radioimmunoassay (RIA) kit (E.I. DuPont de Nemours & Co.; Boston, USA). The antibodies of this kit

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showed specificity for PAF and exhibited no significant cross-reactivity with lyso-PAF (<0.003%). A specific RIA was sensitive over the range of 10 pg/assay. Using polypropylene pipette tips, 100 µl of each test sample and 100 µl of PAF primary antibody were added to a polystyrene tube, which was then incubated at room temperature for 15 min. After the addition of 2 ml of assay buffer (50 mM sodium citrate buffer containing 0.1% sodium azide and 0.05 Tween-20, pH 6.3), the tubes were centrifuged at 2,000 × g for 30 min at room temperature, and the supernatants of tubes were decanted. The remaining radioactivity was counted by a γ-well counter. Concentrations of PAF in the test samples were determined from the calibration curve.

Statistics

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

RESULTS

The time course of the variation in PAF ratio of pre- to post-incubation, when 10^{-8} M PAF was incubated with a piece of human paranasal sinus mucosa, is shown in Figure 1A. PAF concentration was halved within 11.3 min and reduced to as low as 1.6% of the initial concentration in about 60 min. The time course of PAF ratio, when 10^{-8} M PAF was incubated without a piece of human paranasal sinus mucosa, is shown in Figure 1B. PAF was reduced to half within 10.7 min and only 3.4% in about 60 min. Interval comparisons between PAF concentrations in both groups are illustrated in Figure 2. Differences in reduction of PAF concentrations between both groups were estimated at 10, 30, and 60 min. There were no significant differences between the values.

We examined the change of PAF concentration in the medium, when 10^{-8} M lyso-PAF was incubated with a piece of mucosa. The time course is illustrated in Figure 3. A low concentration of PAF (5.0×10^{-11} M) was detected in the medium even at t = 0. The concentration of PAF gradually increased and at 20 min, the mean PAF concentration reached the maximum of 0.36×10^{-8} M. Thereafter, the concentration of PAF decreased to a non-detectable level at 30 min.

DISCUSSION

PAF might be converted to an inactive metabolite in the incubation medium, because the reduction of PAF concentration was observed by RIA. It is known that PAF is metabolized to lyso-PAF by acetylhydrolase (Blank et al., 1981) and lyso-PAF is synthesized to PAF by acetyltransferase (Wykle et al., 1980). It is possible that these converting enzymes exist in the human paranasal sinus mucosa. However, environmental factors, such as temperature and light, are also important for the metabolization of PAF. There was no difference in the reduction of PAF value between the two groups with or without incubation with mucosa, indicating that environmental factors did affect metabolism of PAF in the present study. At 60 min, the concentration of PAF decreased to less than 5% of the initial concentration. The biological activity of PAF in the incubation medium might be also rapidly reduced. PAF could affect the ciliated epithelium for only a short period of time, even if affected. In our previous study (Ganbo et al., 1991), there was no significant difference in the time course of ciliary inhibition between groups of incubation with PAF for 60 or more minutes. In this study, PAF was detected in the medium of lyso-PAF. The concentration of PAF reached the maximum of 0.36×10^{-8} M at 20 min. There was a significant difference between the concentrations at the initial time (t = 0) and at 20 min. These findings suggest that the incubation of lyso-PAF with a piece of mucosa was the trigger of the production of PAF. Moreover, it is presumed that lyso-PAF, a precursor of PAF,

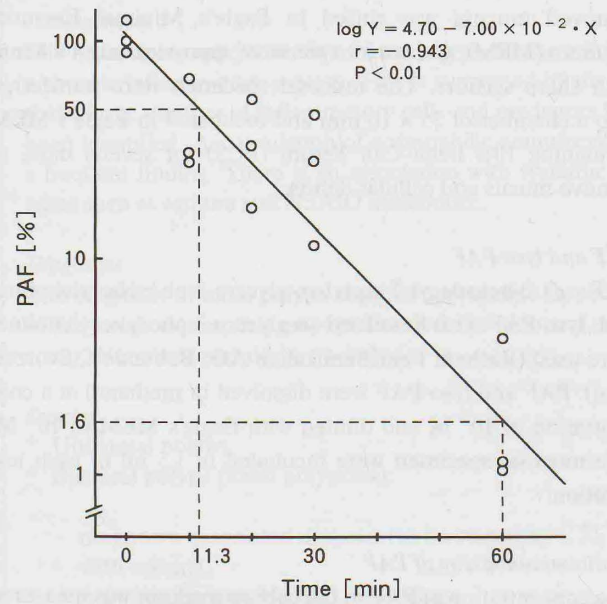

Figure 1A. Time-dependent reduction of PAF concentration in the medium of 10^{-8} M PAF with human paranasal mucosa.

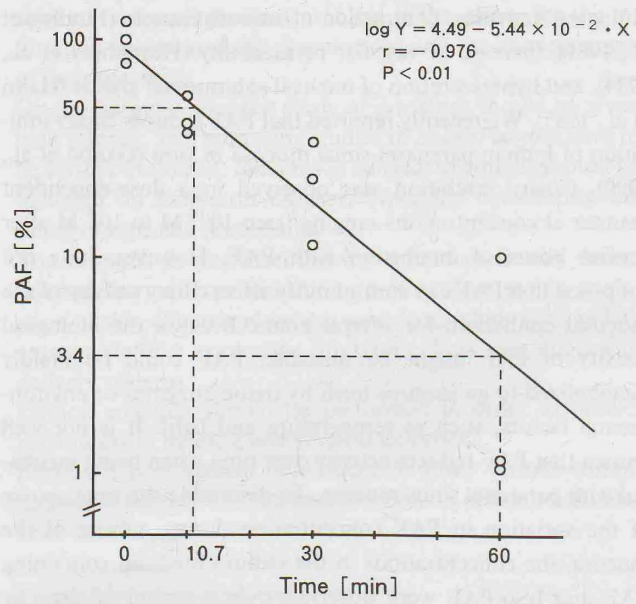

Figure 1B. Time-dependent reduction of PAF concentration in the medium of 10^{-8} M PAF without human paranasal mucosa.

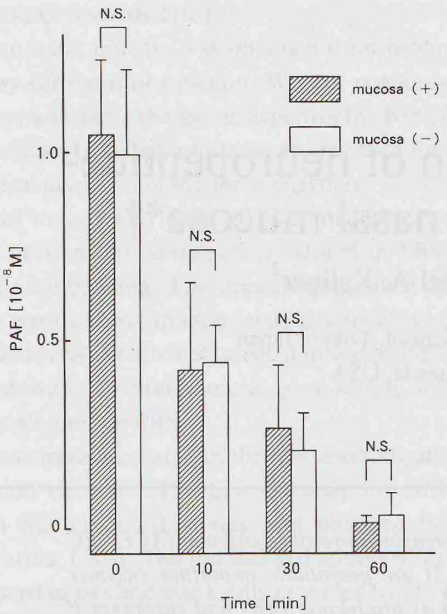

Figure 2. Interval comparisons between PAF concentrations in the respective mediums of 10^{-8} M PAF with and without the mucosa. The values are expressed as mean ± SD.

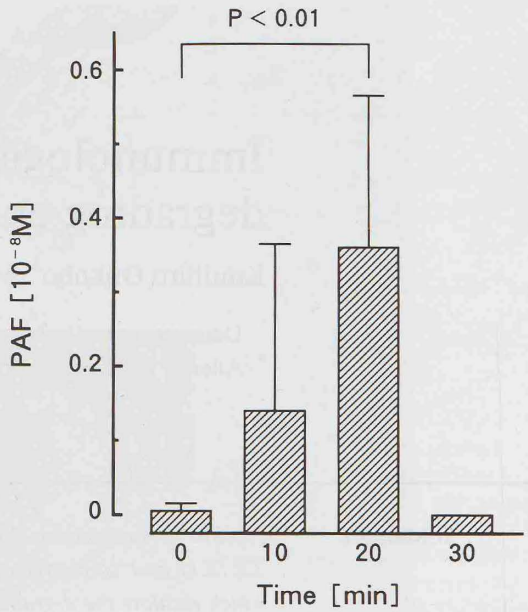

Figure 3. The time course of PAF concentration in the medium of 10^{-8} M lyso-PAF. The values are expressed as mean ± SD.

was converted to PAF in the incubation medium with human paranasal sinus mucosa. However, a low concentration of 5.0×10^{-11} M PAF was detected in the medium even at t = 0. The following two explanations should be considered. First, there was a low concentration of PAF in the medium of lyso-PAF, although the purity of lyso-PAF was over 99%. Secondly, there was little cross-reactivity with lyso-PAF. It was insignificant when the concentration of PAF was measured in the medium of 10^{-8} M lyso-PAF using this PAF RIA-kit. It is a fact that PAF had been produced when lyso-PAF was incubated with a piece of human paranasal sinus mucosa, because there was a significant difference between PAF concentrations at t = 0 and at 20 min. The detailed mechanism is not precisely known, even if the conversion of lyso-PAF to PAF is enzymatically achieved. However, at least, there is a possibility that a converting enzyme, such as an acetyltransferase, might exist in the human paranasal sinus mucosa.

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