Determination of the Platelet Activating Factor in Silicotic Patients and its Effect on Fibroblasts

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Abstract

Platelet-activation factor (PAF), one of the potent proinflammatory mediators, is produced from a large range of cells, including polymorphonuclear neutrophils, monocytes, and natural killer cells. To study the role of PAF in the pathogenesis of silicosis, we determined the PAF in silicotic patients and in healthy persons. The results showed that the concentration of PAF in the plasma of silicotic patients was significantly higher than that of healthy persons. Our *in vitro* experimental results showed that the total numbers of fibroblasts were markedly raised with added PAF from 0 to 1 μ g/ml. Adding 1 μ g/ml PAF significantly increased the total numbers of fibroblasts after culture for 48, 72, 96 hrs. Therefore, we suggest that PAF be possibly involved in the pathogenesis of silicosis. However, the mechanism remains to be further elucidated.

Key words: Platelet activating factor (PAF), Fibroblasts, Silicosis.

Platelet-activation factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent phospholipid proinflammatory mediator that exerts its effects by binding to specific receptors on responsive cell types¹⁻³. Previous studies showed that PAF mediates both the pulmonary microvascular injury and acute inflammatory changes that follow administering endotoxin or bleomycin to rats⁴⁻⁵.

Inhalation of various dusts, such as silica and asbestos results in pulmonary injury and fibrosis. Both *in vivo* and *in vitro* studies have suggested that one mechanism underlying tissue injury induced by these dusts involve the production of mediators by activated lung phagocytes. Furthermore, reactive oxygen species (ROS) have been recognized as injurious to many cell types via modification of macromolecules such as DNA, and the effects of oxidants on cells are complex and dose-related. Oxidants generated by fibrogenic dusts may induce lipid peroxidation, stimulation of cell-signaling cascades and transcription factor, and release of cytokines such as tumor necrosis factor- α ⁶⁻⁷. A number of studies have shown that *in vitro* exposure to O₃ results in increased production and release of PAF by macrophages and

epithelial cells, suggesting that this inflammatory mediator may be important in the actions of O₃ irritant⁸. In macrophages and neutrophils, PAF receptor binding induces a rapid and transient mobilization of intracellular calcium⁹⁻¹¹. This appears to be an important component of the PAF signal transduction pathway leading to increased arachidonic acid release and production of eicosanoids¹²⁻¹⁵ as well as chemotaxis, degranulation, oxidative metabolism, and adherence to vascular endothelium, all responses associated with inflammation. However, there have been no reports on the role of PAF, which is an important inflammatory mediator, in the pathogenesis of silicosis.

The aim of this study was to determine the PAF in the plasma of silicotic patients, and try to find the effect of PAF on the growth of fibroblasts. The data obtained from this study will support some clues for seeking the pathogenesis of silicosis.

Subjects, materials and methods

Subjects

Silicotic patients

There are thirty-one male silicotic patients, aged from 33 to 58 years old, with an average age 45 years. They were divided into 3 categories with 10 category 1 patients, 11 category 2 patients, and 10 category 3 patients. None of these patients had other diseases except silicotic disease. The diagnosis of silicosis was based on a definite history of exposure to silica dust and

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silicotic findings on chest radiography according to the 1980 International Labor Office (ILO) Classification of Radiographs of Pneumoconiosis¹⁶⁾. And all of them were employed in a fluorsparmine company. All the blood samples were collected during a health examination.

Healthy subjects

All the 22 healthy subjects were also male, aged from 25 to 50, with an average of 43 years. Blood samples were collected during a health examination.

Informed consent was obtained from all subjects, and all the subjects were volunteers. The Ethics Committee of Zhejiang Academic of Medical Sciences and the Public Health Department of Zhejiang Province approved the study.

Materials

PAF standard

Sigma Company provided PAF standard.

Cells line

Shanghai Cellular Biological Institute of Chinese Academy of Sciences provided fibroblasts (WI-38 cells).

Method

1. Determination of the concentration of PAF in plasma

The concentration of PAF was measured according to the methods previously described¹⁹⁾. Briefly, 0.5-ml plasma was taken from whole blood after centrifugation; 2 ml of distilled water and 4 ml of methanol were added and mixed, and then 2 ml of distilled water and 2 ml of chloroform were added. Chloroform phase was separated after centrifugation. Then PAF was separated by thin-layer chromatography using activated silica gel G plates developed in a Slovene system, chloroform/methanol/water (65:35:6, vol/vol). The zone that comigrated with PAF standard (Sigma Chemical Co.) was identified by iodine vapor, scraped, and extracted by chloroform/methanol/water (1:2:0.8, vol/vol) as described by Pinckard et al¹⁷⁾. An aliquot was taken to calculate the recovery; another aliquot of the sample was dried and used for PAF assay.

2. Determination of the growth of fibroblasts

Fibroblasts were adjusted to a concentration of 2×10^5 /ml with RPMI-1640, and then 5 ml of cells with a concentration of 2×10^5 were placed in flask. Fibroblasts were cultured in an incubator in a humidified atmosphere of 5%CO₂/95% air at 37 °C.

3. Treatment of cells with PAF

Dose-response: PAF was added to the fibroblasts which were attached in the flask at a final concentrations of 0, 0.01, 0.05, 1, 10, 100 μ g/ml. After culture for 48 hours, 0.25% trypsin was added and the fibroblasts were collected. The cells were counted in a hemacytometer by the conventional method.

Time-effect: 1 μ g/ml PAF was added to the fibroblasts attached in the flask. After culture for 48, 72, 96 hrs, 0.25% trypsin was added to collect the fibroblasts. The cells were counted in a hemacytometer by the conventional method.

Statistical analysis

Value was expressed in terms of mean and standard errors. The differences among groups were tested by one-way analysis of

variance (ANOVA). If a p value was less than 0.05, the difference was considered significant. All the statistics were performed using SPSS 6.1.

Results

1. The concentration of PAF in plasma of healthy and silicotic patients

The concentration of PAF in plasma of categories 1, 2, and 3 of the silicotic patients were 3.309 ± 0.568 ng/ml, 3.197 ± 0.740 ng/ml, and 3.635 ± 0.568 ng/ml, respectively. Although there were no significant differences among the categories, the PAF in silicotic patients was significantly higher than that in healthy persons (1.698 ± 0.534 ng/ml) (Figure 1)

2. Dose-response effect of PAF on the growth of fibroblasts

Figure 2 shows the growth of fibroblasts after adding various doses of PAF. Each dose stimulated the population of fibroblast increase, and there was a dose-related increase after adding PAF from 0 to 1 μ g/ml. However, adding 10 or 100 μ g/ml of PAF showed less growth enhancement than 1 μ g/ml of PAF.

3. Time-response effect of PAF on the growth of fibroblasts

Figure 3 shows the growth of fibroblasts 48, 72 and 96 hrs after adding PAF. The total numbers of fibroblasts in the control and in that with added PAF increased within the experimental period. However, adding 1 μ g/ml PAF more significantly increased the numbers of fibroblast cells than those of the control within the experimental period.

Discussion

Silicosis is a disease induced by inhalation of one of the forms of crystalline silica. A number of studies have demonstrated the relationship of pulmonary silica burden and silicosis. This issue is complicated by a variety of factors, including type of silica particle involved and co-exposures to other types of minerals. A variety of cell types conventionally have been regarded as key participants in the inflammatory process⁷⁾.

Our studies showed that the PAF in the plasma of silicotic patient was higher than that in healthy subjects. This study does not elucidate the mechanism where PAF is synthesized nor the role of PAF in the development and progression of silicotic nodules and fibrosis. However, PAF is now recognized as a

Fig. 1 The concentration of PAF in the plasma of healthy subjects and the different categories of silicotic patients.

* Significantly different from the healthy subjects, $P < 0.05$.

Fig. 2 Numbers of fibroblasts after treatment with different doses of PAF.
Note. Values are mean $\pm$ SE of three experiments
* Significantly different from the control group $P < 0.01$.

Fig. 3 Numbers of fibroblasts after treatment with PAF at different times.
Note. Values are mean $\pm$ SE of three experiments
* Significantly different from the control group $P < 0.01$.

phospholipid synthesized in response to specific stimuli by many target cells in the lung, including the neutrophil, endothelial cells, mast cells, and macrophages, and others. There are substantial data to support the hypothesis that PAF is an important lipid mediator in the inflammatory lung^{1,2}. Intratracheal instillation of PAF induced a dose-dependent acute pulmonary inflammation characterized by an accumulation of macrophages in the alveolar space, as well as degenerative and necrotic changes of alveolar epithelium, and an accumulation of macrophages of polymorphonuclear leukocytes¹⁸. PAF is also involved in bleomycin-induced lung fibrosis and PAF-receptor antagonist ameliorated the fibrosis¹⁹. Furthermore, PAF has been shown to mediate both pulmonary microvascular injury and acute inflammatory changes where an endotoxin was administered to rats²⁰⁻²¹. PAF has also shown to be a potent mediator of increased vascular permeability in the bronchial circulation of the guinea pig, which can be abolished using a PAF-receptor antagonist²². The biological effect of PAF may be amplified by the presence of other mediators released from activated neutrophils and platelets. Such mediators include lysosomal enzymes, toxic oxygen radicals, thromboxane, and leukotrienes^{1,2,5}. Cytokine-mediated interactions are known to regulate the proliferation of fibroblasts under *in vitro* conditions and the monocyte/macrophage figures prominently as an initiator²³⁻²⁴.

Our *in vitro* studies also showed that PAF could increase the fibroblast populations. However, adding 10 or 100 μ g/ml of PAF showed less growth enhancement than 1 μ g/ml of PAF. The reason is still unknown. Probably when the fibroblast was stimulated with a high concentration of PAF, PAF itself has a cytotoxic effect on the fibroblast. As the principal cell type responsible for collagen formation, the fibroblast becomes the

prime focus of attention, and its participation involves functional augmentation in existing cells, with which an increase in population may be associated²⁴. Increase in the fibroblast population may then contribute to connective tissue formation²³. Enhanced growth of fibroblasts and collagen production were promoted by blood monocytes via mediators or by a factor released from human alveolar macrophages after stimulation by a nonmineral²⁵. However, this macrophage-derived growth factor was unable by itself to stimulate fibroblast replication, for which initiating factors provided by fibroblasts or platelets were required to establish competence²⁵. Regulation of fibroblast growth by this means has exposed complexities. And implication of mineral particles in the macrophage regulation of fibroblast proliferation stems from observation on both compact and fibrous dusts. Silica-treated macrophage extract enhanced collagen synthesis under *in vivo* conditions and, reversing the arrangement, an extract from silicotic rat lung stimulated proline incorporation by granulation tissue fibroblasts²⁵. Furthermore, impaired chemotaxis of macrophages by inhaled particles was confined to those that were fibrogenic and proved to be independent of dust burden, but the agent responsible was not identified²³⁻²⁵.

In conclusion, our studies confirmed that PAF is involved with the development of silicosis and can stimulate an increased growth of fibroblasts.

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References

- 1) Spencer DA. Platelet activating factor (PAF). Clin Exp Allergy 1992; 22: 521-4.
- 2) Nojima S. An update on PAF. Lipids 1991; 26: 965-6.
- 3) Stewart AG, Dusting GJ. Characterization of receptors for platelet-activating factor on platelets, polymorphonuclear leukocytes and macrophages. Br J Pharmacol 1988; 94: 1225-

- 33.
- 4) Chang SW, Fewderrsen CO, Henson PM, Voelkel NF. Platelet-activating factor mediates hemodynamic changes in lung injury in endotoxin-treated rats. *J Clin Invest* 1987; 79: 1498-509.
- 5) Evans TW, McAnulty RJ, Rogers DF, Chung KF, Barnes PJ, Laurent GJ. Bleomycin-induced lung injury in the rat: effects of the platelet-activating factor (PAF) receptor antagonist BN 52021 and platelet depletion. *Environ Health Persp.* 1990;85: 65-9.
- 6) Gossart SC, Cambon C, Orfila C, et al. Reactive oxygen intermediates as regulators of TNF-alpha production in rat lung inflammation induced by silica. *J Immunol* 1996; 156: 1540-8.
- 7) Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med.* 1998; 157: 1666-80.
- 8) Cohn LA, Adler KB. Interactions between airway epithelium mediators of inflammation. *Exp Lung Res* 1992; 18: 299-322.
- 9) Block LH, Buhler FR. Atherosclerosis, cell motility, calcium, and calcium-channel blockers. *J Cardiovasc Pharmacol* 1992; 19 Suppl 2: S1-S3.
- 10) Gardner CR, Laskin JD, Laskin DL. Platelet-activating factor-induced calcium mobilization and oxidative metabolism on hepatic macrophages and endothelial cells. *Leukocyt Biol* 1993;53: 190-6.
- 11) Sasaki M, Maeyama K, Watanabe T. Intracellular Ca^{2+} concentration and H_2O_2 production in mouse peritoneal macrophages are stimulated by platelet activating factor. *Lipids* 1991; 26: 1209-13.
- 12) Morris DD, Moore JN. Equine peritoneal macrophage production of thromboxane and prostacyclin in response to platelet activating factor and its receptor antagonist SRI 63-441. *Circ Shock* 1989; 28: 149-58.
- 13) O'flaherty JT, Wykle RL, Miller CH, et al. 1-O-Alkyl-sn-glycerol-3-phosphorylcholines: a novel class of neutrophil stimulants. *Am J Pathol* 1981; 103: 70-9.
- 14) Floch A, Tahraoui L, Sedivy P, Caverio I. The platelet activating factor receptor antagonist, RP 59227, blocks platelet activating factor receptors mediating liberation of reactive oxygen species in guinea pig macrophages and human polymorphonuclear leukocytes. *J Pharmacol Ther* 1991; 258 (2): 567-75.
- 15) Pinckard RN, Jackson EM, Hoppens C, et al. Molecular heterogeneity of platelet-activating factor produced by stimulated human polymorphonuclear leukocytes. *Biochem Biophys Res Commun* 1984; 122: 325-32
- 16) International Labor Office. Guidelines for the use of the ILO International Classification of Radiographs of Pneumoconiosis. Revised edition. Occupational Safety and Health Series. No.22 (Rev 80).Geneva: International Labor Office, 1980.
- 17) Pinckard RN, Farr RS, Hanahan DJ. Physiochemical and functional identity of rabbit platelet activating factor (PAF) released in vivo during IgE anaphylaxis PAF released in vitro from IgE sensitized basophils. *J Immunol* 1979; 123: 1847-57.
- 18) Camussi G, Pawlowski I, Tetta C, et al. Acute lung inflammation induced in the rabbit by local instillation of 1-O-octadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine or of native platelet-activating factor. *Am J Pathol* 1983; 112(1): 78-88
- 19) Giri SN, Sharm AK, Hyde DM, Wild JS. Amelioration of bleomycin-induced lung fibrosis by treatment with the platelet activating factor receptor antagonist WEB 2086 in hamsters. *Exp Lung Res* 1995; 21(2): 287-307
- 20) Suzuki N, Horiuchi T, Ohta K, et al. Mast cells are essential for the full development of silica induced pulmonary inflammation: a study with mast cell-deficient mice. *Am J Respir Cell Mol Biol* 1993; 9: 475-83.
- 21) Chang SW, Federrsen Co, Henson PM, Voelkel NF. Platelet-activating factor mediates hemodynamic changes in lung injury in endotoxin-treated rats. *J Clin Invest* 1987; 79: 1498-1509.
- 22) Evans TW, Chung KF, Rogers DF, Barnes PJ. Effects of platelet-activating factor on airway vascular permeability: possible mechanisms *J Appl Physiol* 1987; 63: 479-84.
- 23) DeLustro F, Mackel AM, DeLustro B, LeRoy EC. Human monocyte regulation of connective tissue growth. *Am Zool.* 1983; 23: 213-20.
- 24) Heppleston AG. Mineral, fibrosis, and the lung. *Environ Heath Persp* 1991; 94: 149-68.
- 25) Bitterman PB, Rennard SI, Hunninghake GW, Crystal RG. Human alveolar macrophage growth factor for fibroblasts. Regulation and partial characterization. *J Clin Invest* 1982; 70: 806-22.