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**Increased platelet-activating factor (PAF)
concentrations in hearts and lungs of Pichinde
virus-infected guinea pigs**

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Summary

Platelet-activating factor (PAF) has been implicated as a cause of cardiopulmonary disturbances in certain diseases. In the present study, concentrations of PAF in hearts, lungs, whole blood, and other organs of control and Pichinde virus-infected guinea pigs on post-inoculation days (PID) 10 and 14 were measured by radioimmunoassay. Results were further confirmed by bioassay after separation and purification with thin-layer chromatography. PAF concentration in the hearts and lungs of virus-infected animals increased significantly on PID 10 and 14, as compared with control levels. PAF level in the blood of infected guinea pigs also significantly increased on PID 14. There was little change of PAF levels in liver and kidney after viral inoculation. Increased PAF concentrations in both hearts and lungs of infected strain 13 guinea pigs suggest that this lipid mediator may play an important role in the development of cardiopulmonary disturbances.

Key words: PAF; Tissue concentrations; Pichinde viral infection

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1. Introduction

Lassa, Argentine, and Bolivian hemorrhagic fevers are serious human diseases caused by arenaviruses. Pichinde virus, a member of the arenaviral family, is non-pathogenic for man, but is lethal to strain 13 guinea pigs, producing a disease resembling human Lassa fever (Jahrling et al., 1981; Peters et al., 1987). Severe cardiopulmonary dysfunctions occurred after Pichinde viral infection (Peters et al., 1987; Liu, 1987; Guo et al., 1990), which might be a determinant of systemic shock at the later stages leading to death of the animals.

Platelet-activating factor (PAF) is recognized as an important lipid mediator in developing circulatory shock, asthma, inflammation, and allergy. Biological activities of PAF include platelet and neutrophil aggregation, hypersensitivity reactions, bronchoconstriction, hypotension, and depression of cardiac contractility (Braquet et al., 1987; Camussi et al., 1990). Because PAF increases cellular release of other mediators of disease, including tumor necrosis factor (TNF) (Rose et al., 1990; Floch et al., 1989), leukotrienes and thromboxane A₂ (Braquet et al., 1987; O'Flaherty et al., 1989), PAF may also be involved in many adverse actions induced by these mediators. Indeed, in Pichinde virus-infected guinea pigs, Liu et al. (1986) demonstrated increased plasma concentrations of sulfidopeptide leukotrienes. Furthermore, there was prolonged survival after daily administration of a leukotriene antagonist (FPL-55712) to the infected animals.

The main objective of this study was to measure PAF concentrations in the blood, hearts, lungs, livers, and kidneys of control and Pichinde virus-infected strain 13 guinea pigs by using radioimmunoassay (RIA) and bioassay. The possible involvement of PAF in the pathogenesis of Pichinde viral infection was also evaluated.

2. Materials and Methods

2.1. Animals

Male strain 13 guinea pigs, weighing 500–600 g, were obtained from our Institute's colony. Guinea pigs were divided into control and two Pichinde virus-infected groups (3–11 animals per group). Animals were infected by the subcutaneous inoculation of 10⁴ plaque-forming units of Pichinde virus (0.2 ml). Experiments were carried out on days 10 and 14 postinoculation (PID), allowing animals to fully develop the disease state; death occurs on days 13–19 (Jahrling et al., 1981).

2.2. Sampling blood and organs

After the guinea pig was anesthetized with Na pentobarbital (40 mg/kg for control, 25 mg/kg for sensitive infected animals), 2 ml of blood was withdrawn from the cannulated common carotid artery into a glass syringe, containing 6 ml of methanol:acetic acid:water (4:0.08:2). Heart, lung, kidney, and liver were quickly removed and placed into liquid nitrogen within 10–20 s.

2.3. Extraction and purification of PAF

Tissue (1–1.5 g) or blood samples were homogenized by an Ultra-Turrax homogenizer (IKA Werk, Germany) in chloroform : methanol (1 : 1). A trace amount of [³H]PAF (26 500 dpm, 56.7 Ci/mmol) (NEN, Boston, MA) was added into the homogenate as a recovery indicator. Total lipids were extracted from blood and tissue by a modification of the method of Bligh and Dyer (1959) with the addition of 2% acetic acid in methanol. PAF in the extracts was separated on layers of silica gel HR (Analtech, Newark, DE) in a solvent system, chloroform/methanol/acetic acid/water (50:35:8:4.5, v/v). The developed thin-layer chromatography (TLC) plates (20 × 10 cm) were exposed to ammonia vapor for about 4 min, sprayed with a solution of 0.05% 2',7'-dichlorofluorescein in 50% ethanol. Patterns of phospholipid separation were viewed under ultraviolet light. The clearly separated gel between phosphatidylcholine (PC) and lyso-PC on the plates was scraped into glass tubes.

PAF was extracted from the gel with the modified Bligh-Dyer method (with acetic acid) to achieve high recovery. After the extracts were evaporated, the residual was reextracted by using the Bligh-Dyer method without acetic acid. A drop of concentrated NH₄OH was added into the system to remove contaminating 2',7'-dichlorofluorescein and silica gel. The extracts were reconstituted in 100 μl of assay buffer (0.1% sodium azide, 0.05% Tween 20 in 50 mM sodium citrate buffer, pH 6.3). 20 μl of the reconstituted extract were counted for radioactivity (LS-5000 model, Beckman Instruments, Fullerton, CA). 40 μl of the extract in assay buffer was taken for RIA, and 10 μl of the same extract was used for bioassay with rabbit platelet aggregation. Recovery of tissue or blood PAF was 80–95% after extraction and purification with TLC.

2.4. PAF radioimmunoassay (RIA)

TLC-purified blood or tissue PAF was measured by using PAF [¹²⁵I]RIA Kit (NEN, Boston, MA). The commercially supplied PAF standards include a mixture of C16-PAF : C18-PAF (1 : 1). Briefly, 100 μl of PAF standards or 40 μl of samples, diluted with 60 μl assay buffer, were incubated with 100 μl of PAF primary antibody in polypropylene tubes for 15 min at room temperature. Then 100 μl of second antibody with [¹²⁵I]PAF were added to the tubes and incubated overnight at room temperature. 2 ml of assay buffer were added to the incubation mixture. The amount of [¹²⁵I]PAF bound to the antibody was measured by centrifuging the antibody complex into a pellet. Radioactivity in the pellet was counted in a 1282 Compugama Universal Gamma Counter (LKB, Wallac Oy, Finland). The concentration of PAF in the samples was determined from the simultaneously determined standard curve (Fig. 1). Values of PAF were corrected by the appropriate dilution factor and percent of recovery.

2.5. PAF bioassay

To evaluate the reliability of the RIA for PAF in tissues, some tissue samples were bioassayed for PAF by rabbit platelet aggregation, described by Bossant et al. (1990). The same mixture of PAF standards for RIA was used for bioassay.

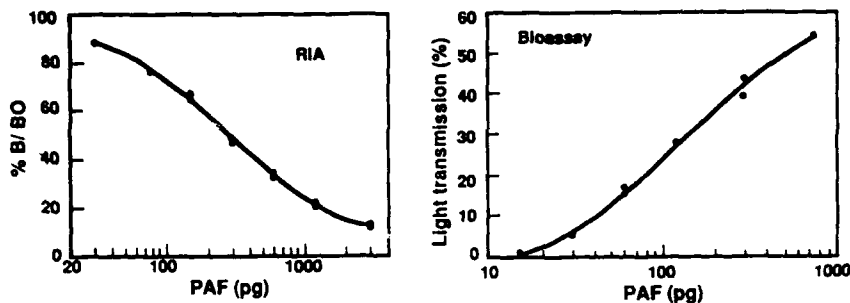


Fig. 1. Typical PAF standard curves established for RIA and bioassay. Data shown represent duplicate measurements.

Platelet aggregation was measured with an aggregometer (430-VS model, Chrono-Log, Havertown, PA).

2.6. Statistical analysis

Data were calculated as means \pm standard error of the mean (SE), and the statistical significance was assessed by the Student *t*-test. Difference between control and infected groups was considered significant at the $P < 0.05$ level. The linear relationship between RIA and bioassay for tissue PAF measurements ($n = 18$) was estimated by the sample correlation coefficient (r).

3. Results

3.1. Comparing PAF values with RIA and bioassay

Fig. 1 shows standard curves of authentic PAF established by RIA and bioassay. The measurable ranges of the two methods were very close (> 0.03 ng). Because only about 1/10 of sample volume ($10 \mu\text{l}$) for RIA was added to measure platelet aggregation, the bioassay was unsatisfactory for measuring very low concentrations of PAF.

PAF concentrations correlated with data from the same guinea pig samples by RIA and bioassay are illustrated in Fig. 2. When results were compared, RIA showed approximately twice the PAF concentration in the same sample as did the bioassay. However, a good correlation ($r^2 = 0.86$, $P < 0.01$) of PAF values was demonstrated while both methods were compared.

3.2. PAF concentrations in blood and organs of anesthetized normal guinea pigs measured by RIA

Normal tissue PAF levels differed among selected organs. The rank order of PAF concentrations (ng/g wet tissue) in the organs was liver (9.8) $>$ kidney

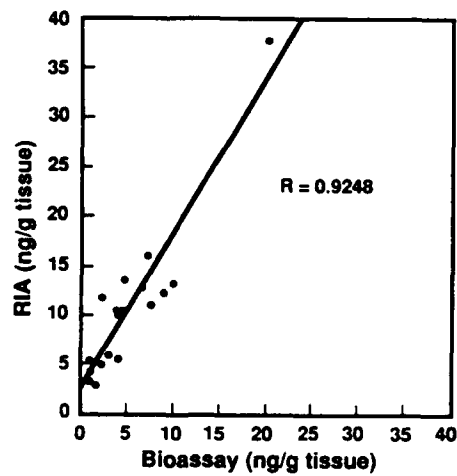


Fig. 2. Correlation of tissue PAF with RIA and bioassay ($n = 18$).

(8.3) > lung (3.8) > heart (2.1) (Fig. 3). The PAF value in whole blood was about 0.33 ng/ml.

3.3. PAF concentrations in blood and organs of Pichinde virus-infected guinea pigs

Compared with control values (2.1 ± 0.3 ng/g wet heart tissue, and 3.8 ± 0.6 ng/g wet lung tissue), PAF concentrations in the infected hearts increased significantly on PID 10 (4.5 ± 0.6 ng/g wet tissue) and PID 14 (5.2 ± 0.4 ng/g wet tissue), and the lung PAF concentration was also significantly augmented in the two infected groups on PID 10 (9.2 ± 1.5 ng/g wet tissue) and PID 14 (17.1 ± 3.9

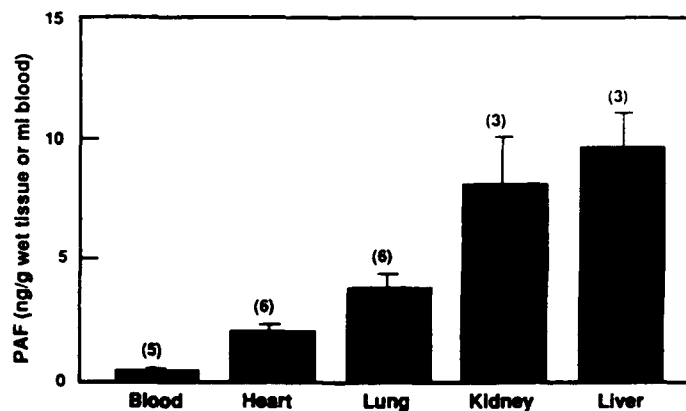


Fig. 3. PAF concentrations (RIA) in the blood and organs of anesthetized normal guinea pigs. Animal numbers appear in the parentheses. Bars represent the mean \pm SE.

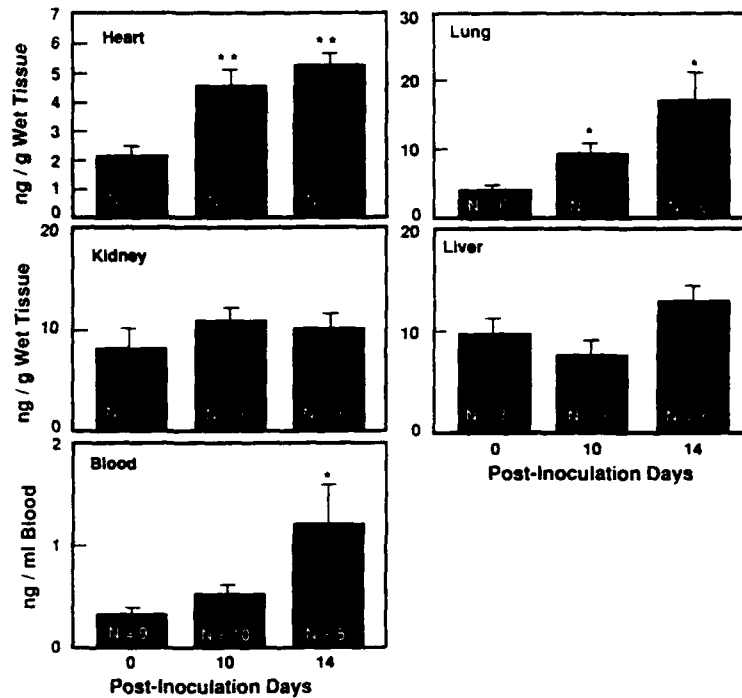


Fig. 4. Pichinde virus-induced changes in PAF levels (RIA) in blood and organs of inoculated guinea pigs. Bars represent the mean \pm SE and significance between control and infected groups was assessed by Student's *t*-test (* $P < 0.05$, ** $P < 0.01$).

ng/g wet tissue). However, there was little change in PAF levels in the infected livers and kidneys. PAF concentrations in blood from infected guinea pigs showed a tendency to increase on PID 10 and differed significantly on PID 14 (Fig. 4).

TABLE 1
PAF CONCENTRATIONS MEASURED BY RIA AND BIOASSAY

	RIA	Bioassay
Heart		
PID 0	2.25 \pm 0.42	< 1.33 ^a
PID 14	4.64 \pm 0.54	1.96 \pm 0.61
Lung		
PID 0	2.51 \pm 0.22	< 1.83 ^a
PID 10	12.03 \pm 0.43	7.17 \pm 1.28
PID 14	11.82 \pm 1.08	4.87 \pm 0.97

Values are expressed as mean \pm SE. $n = 3$ /group.

^a PAF in the tissues of control guinea pigs was too low to be detected by bioassay.

Some samples of heart and lung from control and infected animals were analyzed for PAF concentration by using both RIA and bioassay (Table 1). The RIA showed increase of PAF concentrations in hearts and lungs of infected animals, which were confirmed by bioassay. As noted, PAF concentrations in control heart and lung were too low to be detected by bioassay.

4. Discussion

Increased PAF concentrations in plasma or biological fluids have been reported for several diseases, including endotoxic (Doebber et al., 1985; Chang et al., 1987) and traumatic shock (Stahl et al., 1988), asthma (Fitzgerald et al., 1986), systemic anaphylaxis (Pinckard et al., 1979), persistent pulmonary hypertension of the newborn (Caplan et al., 1990), intestinal ischemia (Filep et al., 1989), and nephrotic syndrome (Egido et al., 1990). PAF, as an autocrine, could be produced in local tissue and exert its bioaction by affecting the same tissue. Recently, a significant amount of PAF was demonstrated in various organs of normal rat including stomach and duodenum (Sugatani et al., 1989), uterus (Yasuda et al., 1986), kidney inner medulla (Woodward et al., 1987), and endotoxin-challenged rat jejunum (Whittle et al., 1987). Bovine brain (Tokumura et al., 1987) and the skin of guinea pigs undergoing irradiation by ultraviolet light (Calignano et al., 1988) also showed high contents of PAF. While the literature has many reports of the pharmacological effects of PAF on the heart, lung, kidney, and liver, little information is available concerning PAF concentrations in these important vital organs in normal and pathological conditions. We are the first to report PAF concentrations in these organs of control and virus-infected guinea pigs.

The PAF RIA kit is designed to measure PAF extracted from blood, serum, plasma, urine, or cell culture media. The development of a high affinity antibody to PAF, combined with a highly specific iodinated PAF tracer, has provided an accurate and very sensitive method. We used the RIA kit to measure PAF concentration in tissues after extraction and TLC purification. PAF values determined by RIA or bioassay correlated well ($r = 0.925$). Because endogenous PAF exists as a mixture of C16 and C18 on the alkyl chain, there is likely to be cross-reactivity of RIA between C16 and C18 PAF (NEN PAF RIA Kit instruction manual). However, results from RIA may reflect total PAF concentration in biological samples. Since the bioassay is less sensitive to PAF with C18 (Bossant et al., 1990), the measured PAF concentrations by the bioassay method can be influenced significantly by the ratio of these analogues. The ratio of C16-PAF and C18-PAF produced in the infected Pichinde virus-infected guinea pigs is unknown. Possibly, an increased C18-PAF concentration occurred postinoculation of virus. This assumption may explain why the measured PAF concentrations with RIA were twice of those with bioassay.

Because PAF is susceptible to plasma acetylhydrolase during sampling and separation of plasma (Hanahan et al., 1985), blood was sampled directly into a methanol solution to destroy acetylhydrolase activity. PAF in whole blood of

normal animals, measured by RIA, was as low as 0.33 ± 0.06 ng/ml. We could not detect blood PAF in normal and most infected guinea pigs by using the bioassay. We demonstrated that PAF concentrations in selected organs of the guinea pig were higher than those in blood, although only a few nanograms per gram of wet tissue. These concentrations were comparable to tissue PAF levels reported in other species (Sugatani et al., 1989; Yasuda et al., 1986; Woodward et al., 1987). Also, PAF levels differed among various organs. The liver and kidney had higher PAF concentrations than the lung and heart.

Our results show that PAF concentrations were significantly increased in hearts and lungs of Pichinde viral infection of strain 13 guinea pigs on PID 10 and 14. The increased PAF concentrations in these two organs may be responsible for the cardiopulmonary dysfunctions of the disease, because PAF is well-known to exert adverse effects on both respiratory and cardiovascular systems (Feuerstein, 1989; Barnes et al., 1989). PAF may result in significant reduction in cardiac contractility and cardiac output (Feuerstein, 1989), sustained bronchial hyper-responsiveness, bronchoconstriction, inflammatory response, and acute lung injury with formation of edema, as well as extravasation of plasma (Barnes et al., 1989).

The cardiopulmonary dysfunctions previously demonstrated in the in-situ or in-vitro studies with Pichinde virus-infected guinea pigs include decreased cardiac output and contractility, right ventricular congestion and dilatation (Peters et al., 1987; Liu, 1987), hyperventilation, increased specific airway resistance, pulmonary edema, terminal shock, and respiratory arrest (Peters et al., 1987; Guo et al., 1990). Since very low concentrations of PAF were detected in normal heart and lung tissues, increased PAF concentrations during the disease appear to have a detrimental impact. Furthermore, we have demonstrated (unpublished observations) that intravenous infusion of C16 PAF (10 ng/min/kg) produced similar cardiopulmonary disturbances as occurred during Pichinde virus infection.

Blood leukocytes and platelets have been demonstrated in-vitro to be important cellular sources of PAF release (Braquet et al., 1987; O'Flaherty et al., 1989). However, numbers of these blood cells were strikingly decreased during Pichinde viral infection (Cosgriff et al., 1987). Thus, the increased blood PAF levels in Pichinde virus-infected guinea pigs may be a result of increased tissue PAF release. We suggest that the elevated PAF concentrations in hearts and lungs, as well as in blood of infected guinea pigs may induce cardiopulmonary disturbances and hypotension, leading to shock at the late stage of the viral disease.

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