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PAF and haematopoiesis: III. Presence and metabolism of platelet-activating factor in human bone marrow

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Abstract

Platelet-activating factor (PAF) is a phospholipid compound with major immunoregulatory activities. The present study shows that human bone marrow contains 576 ± 39 pg PAF/ml ($n = 35$). Bone marrow-derived PAF exhibits the same biophysical and biological properties that synthetic PAF. PAF concentrations in bone marrow are correlated with the granulocyte ($r = 0.4$, $P = 0.02$) but not with the lymphocyte ($r = 0.24$, $P = 0.17$) and the monocyte ($r = 0.12$, $P = 0.48$) counts. In bone marrow PAF is inactivated by a plasma PAF acetylhydrolase activity (48.0 ± 2.3 nmol/min per ml, $n = 34$). Experiments with [^{3}H]PAF indicate that human bone marrow cells actively metabolize this potent molecule by the deacetylation-transacylation pathway. Results of this investigation indicate the permanent presence of significant amounts of PAF in bone marrow suggesting its putative involvement in the processes of bone marrow cell proliferation and maturation.

Keywords: Platelet-activating factor; Bone marrow; Hematopoiesis ; (Human)

1. Introduction

Platelet-activating factor (PAF) is a phospholipidic compound which is produced and acts on a wide range of cells and organs [1–3]. PAF levels are regulated by the enzyme PAF acetylhydrolase which is present in plasma and tissues [4,5]. PAF exhibits potent immunoregulatory activities on blood cells such as T and B lymphocytes, basophils, eosinophils, monocytes and neutrophils [6–11], and enhances the maturation and differentiation of basophils and eosinophils from hematopoietic precursor cells from human umbilical cord blood [12,13]. PAF stimulates DNA synthesis in guinea pig and rabbit bone marrow in vitro [14,15]. Recently we have reported the presence of PAF in femoral bone marrow of rats [16]. Taking all these results into account, we attempted to establish for the first time the presence of PAF and acetylhydrolase activity in normal human bone marrow, to correlate PAF amounts and acetylhydrolase activity with bone marrow cell counts, and to examine PAF uptake and metabolism by bone marrow cells. These data could be of importance for later investigations of the putative role of PAF in the proliferation and maturation processes of human bone marrow cells.

2. Materials and methods

2.1. Patients

This study was performed according to the Helsinki recommendations. Sternum bone marrow samples were harvested from untreated patients referred for diagnosis. All samples used in this study exhibited a normal myelogram. One-ml bone marrow samples were collected on ethanol to assess PAF content. Aliquots of bone marrow samples were used to assess total cellular content using a hemocytometer. Aliquots of bone marrow samples were recovered on EDTA anticoagulated tubes (Vacutainer system, Becton Dickinson, Meylan, France) and centrifuged (400 × g, 10 min). Plasma samples were collected and stored at −80°C until assay of the PAF acetylhydrolase activity. In separate experiments, 1-ml bone marrow samples were recovered on EDTA and centrifuged (400 × g, 10 min, 4°C). Plasma and cell-associated PAF were separately assessed.

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As controls, 1-ml blood samples from healthy volunteers were recovered on ethanol to assess blood PAF levels.

2.2. Processing of samples for PAF determinations

Samples were immediately mixed with four volumes of ethanol (80% final). Lipids were ethanol-extracted during 10 h [17]. Samples were centrifuged ($3000 \times g$, 15 min). Supernatants were dried and recovered with 200 μl of chloroform/methanol (1:1, v/v). Phospholipids were purified on thin-layer chromatography (TLC) plates (Silica gel 60 (20×20 cm, 0.5 mm), Merck). The plates were developed in the mixture of chloroform/methanol/water (70:35:5, v/v) [18]. [^{3}H] PAF was used as marker. Areas of samples on TLC plates with RF values corresponding to the PAF standard were extracted, suspended in 60% ethanol, and assayed for PAF activity.

2.3. PAF assay

PAF activity was measured by platelet aggregation using a Labintec aggregometer [16,19]. Aspirin-treated washed rabbit platelets were stirred in 300 μl Tyrode buffer containing 0.25% gelatin, 1 mM creatine phosphate, and 10 U/ml creatine phosphokinase (pH 7.4). Aggregating activity of the samples were measured using a calibration curve obtained with 2.5 to 20 pg of synthetic PAF (Novabiochem, Switzerland).

2.4. PAF characterization

The lipidic compound extracted from bone marrow was further characterized on the basis of the following criteria: (1) study of the aggregating activity in the presence of 0.1 mM CV 3988 (Takeda Chemical, Osaka, Japan) a specific PAF receptor antagonist [20]; (2) the same after incubation of the samples with phospholipase A_2 from hog pancreas and lipase A1 from *R. arrhizus* [21]; and (3) retention time during TLC [18].

2.5. PAF acetylhydrolase assay

PAF acetylhydrolase activity was assessed according to the method of Miwa et al. with minor modifications [22]. Briefly, 10^5 dpm of 1-*O*-alkyl-2-[^{3}H]acetyl-glycerophosphocholine ([^{3}H]acetyl-PAF; 10 Ci/mmol; NEN), 0.1 mM PAF, Hepes buffer (pH 7.8) in a final volume of 450 μl, and 50 μl diluted plasma (1:50 dilution in Hepes buffer) were incubated for 10 min at 37°C. The reaction was stopped with 100 μl bovine serum albumin (10%) and 400 μl trichloracetic acid (20%). Samples were centrifuged ($1500 \times g$, 15 min) and supernatants were counted in a liquid scintillation counter. Results were expressed as nanomoles PAF degraded per min per ml of plasma (nmol/min/ml) as means of duplicate determinations. The variation between duplicates was less than 5%.

2.6. [^{3}H]PAF uptake and metabolism by human bone marrow cells

Human mononuclear bone marrow cells obtained from heparinized samples and isolated on Ficoll ($280 \times g$; 20 min) were washed with Hanks' balanced salts solution (HBSS) and resuspended in RPMI 1640 without serum (Gibco, France). Cells ($5 \cdot 10^6$ cells/tube) were incubated at 37°C in a volume of 0.5 ml for various periods of time in the presence of [^{3}H]alkyl-PAF (Amersham, France) (final concentration 0.05 nmol/tube; 0.5 μCi) complexed to human serum albumin (final concentration 2 mg/ml). Experiments were performed in quadriplicate. One set of duplicates was used to quantify uptake and the other to quantify metabolism.

To assess PAF metabolism incubations were terminated by the addition of methanol/chloroform (2:1, v/v) to the reaction mixture, and the lipids were extracted by the method of Bligh and Dyer [23]. In these experiments the labeled products recovered include both those in the medium and those associated with the cells. Recovery of added radioactivity after lipid extraction was 85%. The labeled compounds derived from [^{3}H]alkyl-PAF were separated using TLC plates (Silica Gel 60 (20×20 cm, 0.25 mm), Merck, France) developed in chloroform/methanol/acetic acid/water (50:25:8:4, v/v) [24]. Each lane was divided in areas of 0.5 cm length which were scraped into vials and radioactivity was measured on a Packard liquid scintillation counter. Unlabeled PAF (1-*O*-alkyl-2-acetyl-glycerophosphocholine), lyso PAF (1-*O*-alkyl-2-lyso-glycerophosphocholine), phosphatidylcholine (1-*O*-alkyl-2-acyl-glycerophosphocholine) and neutral lipid (1-*O*-alkyl-2-acetyl-glycerol) were used as standards and were visualized with iodine vapour.

2.7. Statistical analysis

Results are reported as mean $\pm$ S.E. or as individual data. Correlations between PAF level, acetylhydrolase activity and cell counts were calculated by linear regression analysis.

3. Results

We first investigated the presence of PAF in human sternum bone marrow samples. As shown in Fig. 1, lipids extracted from all bone marrow specimens exhibit a PAF-like activity with a mean level of 576 ± 39 pg PAF/ml ($n = 35$). Bone marrow PAF amounts are significantly elevated ($P = 0.0001$, Mann–Whitney U test) as compared to blood PAF amounts in healthy controls (374 ± 22 pg PAF/ml, $n = 24$). The plasma- and cell-associated

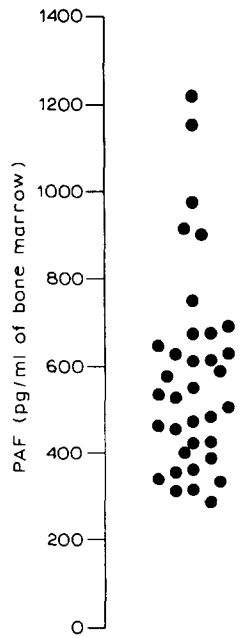

Fig. 1. PAF in human bone marrow. Results are expressed in picograms PAF per ml of bone marrow (individual data).

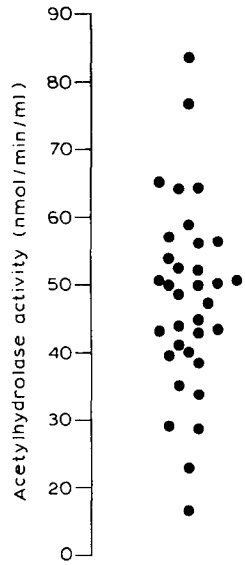

Fig. 3. PAF acetylhydrolase activity in plasma of human bone marrow. Results are expressed in nanomoles PAF per min per ml of plasma (individual data).

PAF have been investigated. Results show that 58.8 ± 1.6% ($n = 10$) of the PAF in human bone marrow is cell-associated. The PAF-like material recovered from bone marrow samples has biological and physicochemical characteristics identical to those of authentic PAF: (1) it induces the aggregation of washed rabbit platelets that are refractory to arachidonic acid and ADP-mediated pathways (Fig. 2); (2) The platelet aggregating activity is totally inhibited by the PAF antagonist CV 3988 (Fig. 2); The aggregating activity is sensitive to phospholipase A_2 but not to lipase A1 (data not shown); (3) The PAF-like material exhibits on TLC a retention time similar to that of synthetic PAF (data not shown). These results indicate that the material recovered from human bone marrow samples is PAF.

In order to investigate the putative cellular origin of the bone marrow-derived PAF, correlations were performed between bone marrow PAF contents and the counts of erythroblasts, granulocytes, monocytes and lymphocytes.

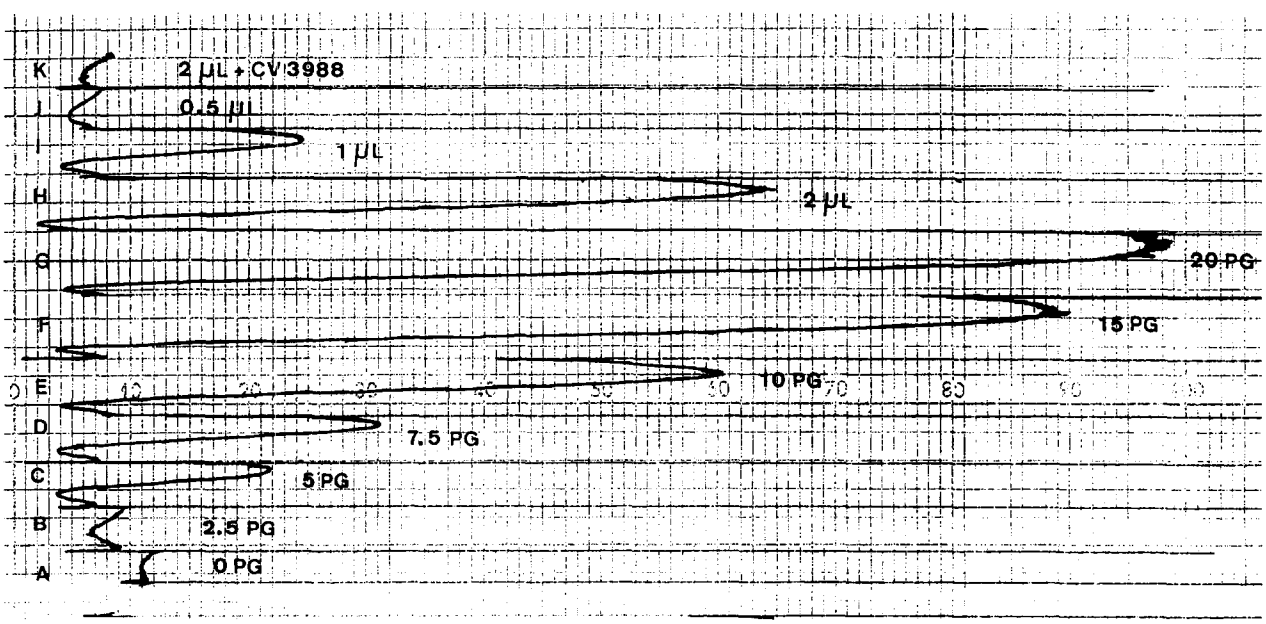

Fig. 2. Representative tracing of platelet aggregation induced by synthetic and bone marrow-extracted PAF. (A–G) Dose-response of platelet aggregation induced by synthetic PAF. (H–J) Dose–response of platelet aggregation induced by PAF extracted from human bone marrow. (K) 2 µl of the extract in the presence of CV 3988 (0.1 mM) a specific PAF receptor antagonist.

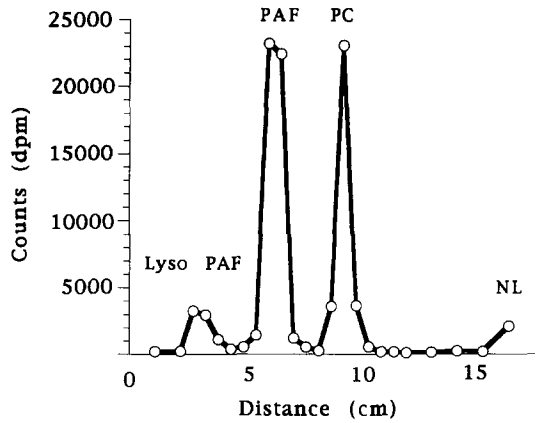

Fig. 4. Distribution of label in products after incubation of [^{3}H]alkyl-PAF with human bone marrow cells for 60 min at 37°C. PAF, 1-*O*-alkyl-2-acetyl-glycerophosphocholine; Lyso PAF, 1-*O*-alkyl-2-lyso-glycerophosphocholine; PC, 1-*O*-alkyl-2-acyl-glycerophosphocholine; NL, neutral lipid. One representative tracing of five independent experiments is shown.

Significant correlations were found between PAF amounts and granulocyte counts ($r = 0.4$, $P = 0.02$) but not with erythroblast ($r = 0.14$, $P = 0.4$), lymphocyte ($r = 0.24$, $P = 0.17$) and monocyte ($r = 0.12$, $P = 0.48$) counts.

We also investigated the presence of a PAF acetylhydrolase activity in human bone marrow plasma samples. As shown in Fig. 3, a PAF acetylhydrolase activity is detected in all bone marrow plasmas at the mean levels of 48.0 ± 2.3 nmol PAF/min/ml ($n = 34$). No correlation is documented between PAF levels and acetylhydrolase activity ($r = 0.09$, $P = 0.58$). No correlation is documented between PAF acetylhydrolase activity and the various cell counts (data not shown).

In the next set of experiments bone marrow mononuclear cells were incubated with labeled PAF for 5 min to 3 h after which uptake and metabolism were assessed. After

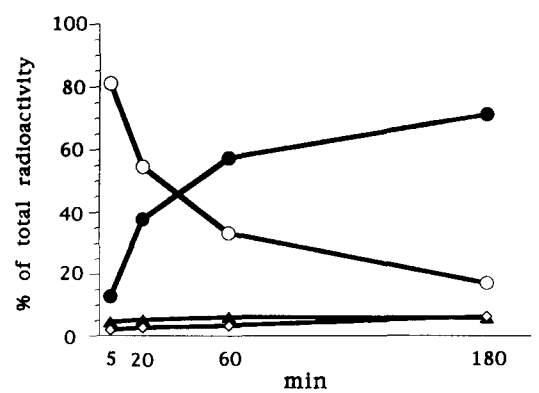

Fig. 5. Time-course of the conversion of 1-*O*-[^{3}H]alkyl-2-acetyl-glycerophosphocholine (○) to 1-*O*-[^{3}H]alkyl-2-acyl-glycerophosphocholine (●), 1-*O*-[^{3}H]alkyl-2-lyso-glycerophosphocholine (▲), and [^{3}H]-neutral lipids (◇) in human bone marrow cells. Results are expressed as the percentage of total radioactivity recovered following incubation of bone marrow cells with [^{3}H]PAF using the conditions described in Section 2. The values are the mean of five independent experiments.

5, 10, 20 and 60 min of incubation bone marrow cells incorporate 51, 62, 70 and 76% of the initially added radioactivity ($n = 3$). A representative TLC profile of labeled metabolites of PAF is reported in Fig. 4. When [^{3}H]PAF is incubated with bone marrow cells for 1 h the label migrates with PAF and phosphatidylcholine (i.e. with a long chain acyl group at the *sn*-2 position of the glycerol backbone). The distribution of label as a function of time is reported in Fig. 5. At each time point, less than 6% of the radioactivity migrates with lyso PAF or neutral lipids. The time-dependent decrease of [^{3}H]PAF is paralleled with the time-dependent increase of [^{3}H]phosphatidylcholine. Incubations of [^{3}H]PAF for 3 h in the absence of bone marrow cells result in no appreciable catabolism (data not shown).

4. Discussion

The present work reports the presence of PAF and PAF acetylhydrolase activity in human bone marrow and the catabolism of PAF by human bone marrow cells. The platelet aggregation method is a sensitive assay that can be used to detect picogram quantities of PAF in biological samples [25]. Lipid extraction and TLC purification before assay ensure the accuracy of the results [26]. Furthermore, we use several experimental biological and physicochemical criterias to demonstrate that the PAF-like material recovered from human bone marrow was PAF.

In this study PAF was detected in all human bone marrow samples tested with a mean level of 576 ± 39 pg PAF per ml, i.e., significantly elevated as compared to blood PAF levels (374 ± 22 pg per ml). At this time we cannot firmly exclude that circulating blood PAF does not account for a small part of the PAF found in human bone marrow. The PAF acetylhydrolase activity in bone marrow plasma (48.0 ± 2.3 nmol PAF/min per ml, $n = 34$) is rather similar to the one previously reported in human plasma [27–29].

The cellular origin of the bone marrow-derived PAF remains an open question. While PAF amounts are not correlated with lymphocyte, monocyte and erythroblast counts, a significant relation is documented between PAF levels and granulocyte counts. Interestingly, granulocytes produce PAF after in vitro stimulation [17,30,31] while the release of PAF from normal human lymphocytes is unsuccessful [17]. At this time the production of PAF by erythroblasts is not documented. After in vitro stimulation of blood cells most of the newly synthesized PAF remains cell-associated [30–33]. We find that only 60% of the PAF in human bone marrow is cell-associated. Unusually among human blood cell types, monocytes are reported to release significant amounts of their newly synthesized PAF [34]. However, there is no relation between PAF amounts and monocyte counts. We may thus suggest that a part of the PAF found in bone marrow may originate from another

population such as epithelial and dendritic cells and/or fibroblasts. Of interest, human skin fibroblasts produce PAF in vitro and release half of their newly synthesized PAF [35]. This hypothesis is also suggested to account for the high amounts of PAF present in human thymus [36]. Clearly the capacity of human bone marrow cells to produce PAF deserves to be investigated.

The catabolism of PAF is an effective mean by which cells can regulate its level. PAF is metabolized by numerous cells using the deacetylation–transacylation pathway. Accumulation of the deacetylated compound lyso PAF is found in human endothelial cells [37] and rat alveolar macrophages [38]. By contrast, in human monocytes [39], neutrophils [24] and lymphocytes [40] the major PAF metabolite is 1-*O*-alkyl-2-acyl-glycerophosphocholine. Results of the present study indicate that human bone marrow cells metabolize PAF using the deacetylation–transacylation pathway. Similarly to that found for human neutrophils [24] and lymphocytes [40], the metabolite lyso PAF, which appears briefly in the system, is quikly reacylated with a fatty acid at the 2 position of the PAF glycerol backbone. The temporal relationships between the deacetylation and reacylation of PAF found in human bone marrow cells is in agreement with those found in human neutrophils and lymphocytes [24,40]. Thus, PAF is similarly metabolized in bone marrow and in peripheral blood.

Is the 500 pg of PAF detected per ml of human bone marrow physiologicaly significant? High amounts of PAF are usually added to cell cultures because PAF is rapidly catabolized in the culture medium by serum or cellular PAF acetylhydrolase. Kato et al. report that 55 μg of PAF and 55 ng of a non-metabolizable PAF agonist enhance [^{3}H]thymidine incorporation in freshly isolated guinea pig bone marrow cells [14]. Saito et al. report that 550 pg of PAF enhance both eosinophilic and basophilic differentiation in human hematopoietic precursor cells [12]. Finally, pM amounts of PAF induce interleukin-1 and tumor necrosis factor production from human blood monocytes [41,42]. With respect to the plasma acetylhydrolase activity detected in human bone marrow and the capacity of bone marrow cells to metabolize PAF, we suggest a continuous local PAF synthesis in the bone marrow. The permanent presence of PAF in bone marrow may be compared to a continuous stimulation with low doses of PAF. The capacity of PAF to modulate the processes of human bone marrow cell proliferation and maturation is presently under investigation.

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