Simultaneous Presence of Platelet Activating Factor, Leukotriene B₄, Prostaglandin F₁₂ and F₂ₓ in the Supernatant of Human Neutrophils Treated with Phospholipase A₂ of Human Monocytes

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Summary. The simultaneous presence of platelet-activating factor, leukotriene B₄, prostaglandin F₁₂ and F₂ₓ are detectable in the supernatant of human neutrophil granulocytes treated with phospholipase A₂ of human monocytes. This enzyme is suspected to play an important role in the pathomechanism of inflammation.

Key words: Human neutrophil granulocyte – Leukotriene B₄ – Phospholipase A₂ – Platelet-activating factor – Prostaglandin F₁₂, F₂ₓ

Introduction

In our laboratory the phospholipase A₂ (PLA₂) with a molecular weight of 73 kD and i.p. of 8.2 (PLA₂-73) has been described (Sipka et al., submitted). This enzyme derives from human mononuclear cells, presumably from monocytes, and represents a new type of human phospholipases A₂, not previously known [9, 10]. We had earlier observed that this enzyme can induce the production of platelet-activating factor (PAF) in the suspension of human neutrophils (Sipka et al., submitted) and can cause a suppression of the random migration of these cells (Sipka et al., submitted). The production of PAF requires the activation of PLA₂ in the leukocytes [7]. At the same time, PLA₂ produces arachidonic acid as well [5]. The derivatives of arachidonic acid, especially prostaglandin F₁₂ and F₂ₓ (PGF₁₂, PGF₂ₓ) and leukotriene B₄ (LTB₄) are potent mediators of inflammation, as is PAF [4, 8]. In a further series of experiments, we observed PGF₁₂, PGF₂ₓ, and LTB₄ in the suspension of human neutrophils treated with PLA₂-73 [6]. In this study, we demonstrate that all the previously mentioned mediators, PAF, PGF₁₂, PGF₂ₓ, and LTB₄ can be simultaneously present in the suspension of human neutrophils treated with PLA₂-73.

Materials and Methods

Preparation of Cells

Heparinized venous blood was obtained from healthy volunteers. Mononuclear cells and neutrophils were separated using Ficoll (Pharmacia Fine Chemicals, Sweden) and Uromiro (Bracco Industria Chimica, Italy) density centrifugation followed by dextran sedimentation of the neutrophil-rich pellet. Residual erythrocytes were lysed with hypotonic saline. The neutrophil granulocytes were washed and suspended in TC-199 media [2]. Their purity was above 95%.

Preparation and Identification of Phospholipase A₂

Preparation and identification was carried out according to our previous description (Sipka et al., submitted).

Identification of Platelet-Activating Factor (PAF)

Mass spectra of PAF were recorded by VG-7035 mass spectrometer (VG Analytical, England) using the DCI (Desorption Chemical Ionisation) technique [11]. The reagent gas was NH₃ at 10³ Pa, ion energy: 50 eV, ion source temperature: 100°C, DCI emitter current: 1 A, heating rate: 20 s to 1 A and resolution: 1,000. Various samples of 1 ml were saturated by NH₄Cl. Aliquots of 50 μl were dried on the DCI probe tip.

Identification of Prostaglandin Derivatives and Leukotriene B₄

Thermospray-high performance liquid chromatography-mass spectrometry (TSP-HPLC-MS) was carried out [1] in order to detect PGF₁₂, PGF₂ₓ, and LTB₄ in the supernatants of neutrophils treated with PLA₂-73.
Fig. 1. Mass spectrometric characteristic peaks of PAF. PAF standard solution ($5 \times 10^{-6}$ mol/ml, above) and the supernatant of human neutrophils treated (cultured) with PLA$_2$-73 ($10^{-8}$ mol/ml, below). The molecular peaks (M$^+$) of PAF were found at m/e 523 ($n=15$) and m/e 537 ($n=16$). Other peaks at m/e 524 ([M + H]$^+$), m/e 541 ([M + NH$_4^+$]), and m/e 546 ([M + Na]$^+$) can also be detected. The peak at m/e 436 is due to the loss of choline from the molecular ion [11].

Fig. 2. TSP-HPLC-MS total ion current profile of a supernatant of human neutrophil granulocytes treated with PLA$_2$-73