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Movement of monoglyceride derived from hydrolysis of fluorescence-labeled lyso platelet-activating factor by lysophospholipase C through plasma membranes of porcine kidney epithelial cell line LLC-PK1

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Abstract

To investigate the mechanisms of the release of lyso platelet-activating factor (PAF), an alkyl ether-linked lysophosphatidylcholine, from the kidney epithelial cell line LLC-PK1, the cell monolayer was incubated with a fluorescence-labeled lysoPAF analog, Bodipy-lysoPAF, on either the basolateral or apical side. The fluorescent lipids in the culture media mixed with or without bovine serum albumin at a final concentration of 2% were analyzed by thin layer chromatography. In both cases, two major bands, assignable to Bodipy-lysoPAF and Bodipy-monoglyceride (MG), were detected in the culture medium to which Bodipy-lysoPAF had been added, whereas the culture medium at the opposite side exhibited only the major band of Bodipy-MG. Our results suggest that lysoPAF was degraded by high ecto-lysophospholipase C activity. The possible physiological significance of this metabolic pathway is discussed.

Keywords: Lyso platelet-activating factor; Lysophospholipase C; Monoglyceride; Lipid transport; Kidney tubule cell

1. Introduction

Human and animal urine is known to contain measurable amounts of platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) [1,2]. In an early investigation to examine the origin of urinary PAF, only 0.29% of [^{3}H]PAF injected into the renal artery of isolated rat kidney perfused with a cell-free medium containing 1% bovine serum albumin (BSA) was recovered in the urine [3]. About 70% was recovered in the renal tissues as lysoPAF (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine), monoglyceride (MG, alkyl-glycerol), phosphatidylcholine (PC, alkyl-acyl-glycerophosphocholine) and diglyceride (DG, alkyl-acyl-glycerol) [3]. These results suggest that circulatory PAF is effectively degraded by the processes of glomerular infiltration and re-uptake in the renal tubular epithelium, although urinary excretion of platelet-activating factor in children with hemolytic uremic syndrome was significantly higher than in controls [4]. LysoPAF, an alkyl ether-linked lysophosphatidylcholine with chemotactic activity on human monocytes [5], was metabolized to MG and PC in isolated rabbit glomeruli [6]. Secretion of PAF was mediated by

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multidrug-resistant 1 P-glycoprotein in cultured human mesangial cells [7]. However, little is known about how PAF and lysoPAF are transported through renal tubules into the urine or blood, as well as metabolic pathways for exogenous phospholipids in the renal tubule system.

Using albumin back-extraction procedure, we previously monitored the transbilayer movement of radioactive PAF, lysoPAF and metabolically stable PAF analogs across the plasma membranes of platelets [8,9], neutrophils [8], HL-60 cells [10] and K562 cells [10,11]. In this study using albumin-back extraction, we used a fluorescent-labeled lysoPAF to examine whether lysoPAF is transported through the monolayer of cultured porcine proximal renal tubule epithelial cells (LLC-PK1) with or without metabolic degradation.

2. Materials and methods

2.1. Materials

Bodipy-lysoPAF, 1-(*O*-11-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl)amino)undecyl)-*sn*-3-glycero-3-phosphocholine, and Dulbecco's modified Eagle's medium (DMEM) without phenol red were obtained from Invitrogen (Tokyo, Japan). Tricyclodecane-9-yl-xanthogenate (D609), fluorescein isothiocyanate-labeled dextran (FITC-dextran, average molecular weight 4000), fatty acid-free BSA, 1-*O*-hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (C_{16}-lysoPAF), *sn*-glycero-3-phosphocholine (GPC) and Medium 199 were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitin and 1-*O*-hexadecyl-*sn*-glycerol were from Funakoshi Co. (Tokyo, Japan). Sodium *ortho*-vanadate was from Wako Pure Chemicals (Osaka, Japan). Fetal calf serum was from ICN Biochemicals (Aurora, OH, U.S.A.).

2.2. Cell culture

LLC-PK1 cells, a porcine-derived proximal renal tubule epithelial cell line, were obtained from the Japan Health Sciences Foundation (Osaka, Japan). The cells were cultured in Medium 199 supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere containing 5% CO_2 and subjected to passage every 3 days, as reported previously [12].

2.3. Assay of permeability of cell monolayers

The permeability of the LLC-PK1 cell monolayer on 3.0 μm polycarbonate filter supports (I.D., 24 mm, Transwell^{TM}, Costar, Tokyo, Japan) by FITC-dextran was assayed as described [13]. The cell monolayer was pretreated with 0.1 μM or 1 μM C_{16}-lysoPAF for 10 min. Then, FITC-dextran at a final concentration of 50 μg/ml was added to the medium in the upper chamber, followed by incubation at 37 °C for 1 h. The amount of FITC-dextran that passed through the cell monolayer to the medium in the lower chamber was measured with the use of a multi-label counter (Wallac 1429 ARVOsx, Perkin-Elmer, Tokyo, Japan).

2.4. Uptake experiments

For uptake experiments, LLC-PK1 cells were seeded onto polycarbonate filter supports (I.D., 24 mm; Transwell^{TM}) at a density of 1×10^6 cells/well (2.1×10^5 cells/cm^2). The cells reached confluence after 3–5 days of incubation. The cells were rinsed with phosphate-buffered saline twice 3 h before experiments, and incubated with DMEM without phenol red and fetal calf serum for 3 h. The volume of medium in the each chamber of the Transwell^{TM} filter supports was 2 ml. Bodipy-lysoPAF dissolved in 0.02 ml of saline containing 0.1% BSA was added to the medium in the upper chamber (apical medium) or lower chamber (basolateral medium) at a concentration of 1 μM. After 1 h incubation at 37 °C, the albumin back-extraction procedure was performed, essentially as described previously [9]. In brief, the apical and basolateral media of the cell culture were incubated with 0.2 ml of phosphate-buffered saline containing 20% BSA for 10 min to extract fluorescent lipids located in the outer half of the lipid bilayer of the cell membranes. The final concentration of BSA was 2%. The medium in each chamber was collected. The remaining cells were treated with 0.05% trypsin for 10 min at 37 °C, and collected by washing the filter with 1 ml of phosphate-buffered saline.