Structure-activity relationship of lysophosphatidylcholines in HL-60 human leukemia cells[1]

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KEY WORDS lysophosphatidylcholines; leukemia; calcium signaling; structure-activity relationship; platelet activating factor

ABSTRACT

AIM: To explore the structure-activity relationship of lysophosphatidylcholine (LPC) and lysolipid molecules from a marine sponge and ladybirds. **METHODS**: We tested three synthetic LPCs and four natural lysolipids on Ca^{2+} mobilization in HL-60 human leukemia cells. **RESULTS**: We observed lysolipid-mediated Ca^{2+} mobilization. The activity was the same in both ester- and ether-linked lysolipids, and introduction of a double bond or methoxy group on the alkyl chain did not significantly modulate the activity. However, replacement of trimethylammonium moiety in the choline structure with ammonium moiety reduced the activity. Furthermore, change of the alkyl chain length influenced the Ca^{2+} response. **CONCLUSION**: LPC-induced Ca^{2+} mobilization might be dependent on the length of alkyl chain and the presence of choline moiety in HL-60 leukemia cells.

INTRODUCTION

Recently, a series of lipid mediators containing a lyso-type structure has drawn attention by the molecular cloning of their receptors in the plasma membrane[1,2]. Lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), lysophosphatidylcholine (LPC), and sphingosylphosphorylcholine (SPC) are the representatives of intercellular lipid mediators. They are devoid of a lipid chain on the *sn*-2 carbon in phospholipids or sphingolipids[1,2]. Nine receptors for LPA and S1P, and three receptors for LPC and SPC have been reported[2-4].

Lysophosphatidylcholine (LPC, 1-palmitoyl-*sn*-glycero-3-phosphocholine) is an intercellular mediator regulating cell functions such as cell growth and immune response[1,5-10]. A glycerol in the LPC structure forms a linker between a fatty acid chain and a phosphorylcholine moiety (Fig 1). Previously, we isolated lysolipids from a marine sponge. The lysolipids are cytotoxic in cancer cells and have inhibitory effects on cholesterol synthesis in human liver cells[11,12]. In this study, we aimed to elucidate the action mechanism of LPC and lysolipid molecules from a marine sponge and ladybirds by testing the effect of three synthetic LPCs and four natural lysolipids on Ca^{2+} mobilization in HL-60 human leukemia cells. Structure-activity relationship of lysophosphatidylcholines on Ca^{2+} mobilization was established for the first time.

MATERIALS AND METHODS

Materials Compounds A-C were isolated from

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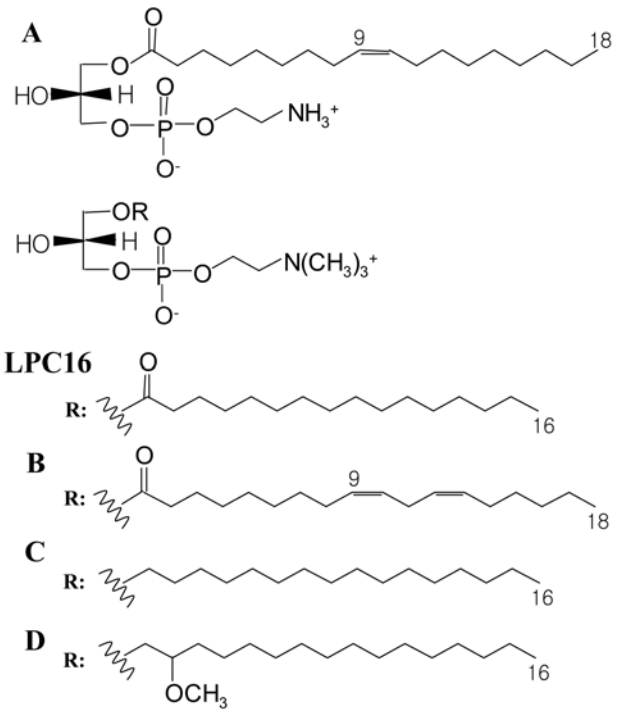

Fig 1. Structures of 16:0 LPC and compounds A-D.

the Coccinellid Beetles *Harmonia axyridis* and *Aiolocaria hexaspilota*. Compound D was isolated from a marine sponge of *Stelletta sp*[11,12]. 1-Stearoyl ($C_{18:0}$) LPC, 1-palmitoyl ($C_{16:0}$) LPC, and 1-myristoyl ($C_{14:0}$) LPC were purchased from Avanti Polar lipids (Alabaster, AL, USA); Fura-2/acetoxymethyl ester was from Calbiochem (Darmstadt, Germany).

Cell culture HL-60 cells were maintained in Modified Eagle's Medium containing 10 % (v/v) fetal bovine serum, benzylpenicillin 100 kU/L, streptomycin 50 mg/L, glutamine 2 mmol/L, and sodium pyruvate 1 mmol/L at 37 ºC in a humidified 5 % CO_2 incubator.

Measurement of intracellular Ca^{2+} concentration The cells were sedimented, resuspended with Hepes-buffered medium consisting of Hepes 20 mmol/L, pH 7.4, NaCl 103 mmol/L, KCl 4.8 mmol/L, KH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $CaCl_2$ 0.5 mmol/L, $NaHCO_3$ 25 mmol/L, glucose 15 mmol/L, and 0.1 % bovine serum albumin (fatty acid free), then incubated with Fura-2/acetoxymethyl ester 5 μmol/L for 40 min. Fura 2-loaded cells were washed twice with the Hepes-buffered media and resuspended in the same media. Fluorescence emission at 510 nm wavelength from two excitation wavelengths (340 nm and 380 nm) were measured every 0.1 s (F4500, Hitachi, Japan). The ratio of fluorescence intensities from the two wavelengths was monitored as an estimate of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).

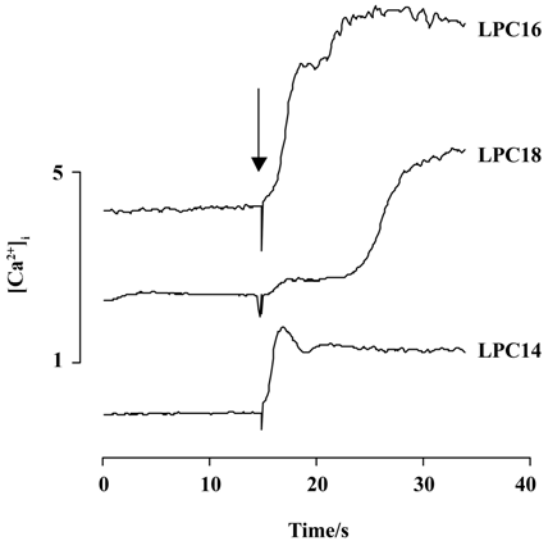

Fig 2. Representative Ca^{2+} traces with 10 μmol/L of 14:0 LPC, 16:0 LPC, and 18:0 LPC.

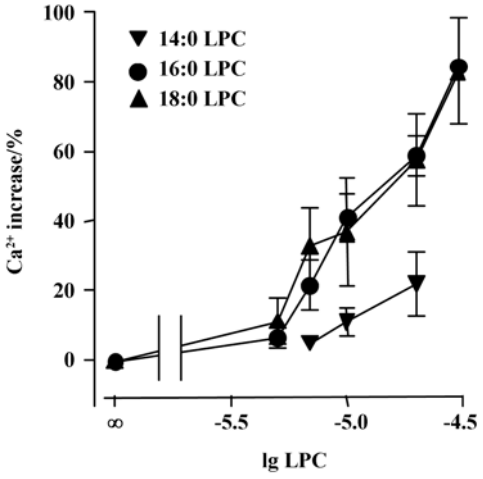

Fig 3. Concentration-response curves of LPC-induced Ca^{2+} mobilization in HL-60 cells. Maximum Ca^{2+} increase by each concentration of 14:0 LPC , 16:0 LPC , or 18:0 LPC was converted to percentage to digitonin-mobilized Ca^{2+} increase. n=3-5 independent experiments. Mean±SD.

Data analysis The representative traces for $[Ca^{2+}]_i$ were chosen out of 3-5 separate experiments .

RESULTS AND DISCUSSION

LPC mobilizes Ca^{2+} in HL-60 cells In the present study, we used three synthetic LPCs and four lysolipid analogues from a marine sponge and ladybirds. They included 14:0 LPC (myristoyl), 16:0 LPC (palmitoyl), 18:0 LPC (stearoyl), 1-*O*-(9'*Z*-octadecanoyl)-*sn*-glycero-

3-phosphoethanolamine (Compound A), 1-*O*-(9'*Z*,12'*Z*-octadecanoyl)-*sn*-glycero-3-phosphocholine (Compound B), 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (Compound C), and 1-*O*-(2'-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine (Compound D). Their structures are shown in Fig 1. The structure of LPC is composed of 3 different parts. Glycerol, a backbone of glycerolipids, is a linkage component, which bridges a hydrophobic lipid chain part and a phosphorylcholine part. Compound A has an ethanolamine instead of the choline. Compounds A and B have unsaturation on the acyl chain. Compounds C and D have an ether linkage instead of an ester bond in the LPC structure (lyso-PAF). Compound D has a methoxy group on C-2 of the alkyl chain. The lengths of the hydrophobic alkyl chain are either 16 or 18 except 14:0 LPC (Fig 1).

Okajima *et al* reported LPC induced increase of intracellular Ca^{2+} concentration in HL-60 cells[13]. We reproduced the response of LPC. As shown in Fig 2, 14:0, 16:0, and 18:0 LPCs induced Ca^{2+} increase in HL-60 cells. Concentration-response curves are shown in Fig 3. Ca^{2+} responses observed in our study were in agreement with the results of Okajima *et al*. 18:0 LPC did not induce Ca^{2+} increase significantly and 14:0 LPC was equipotent to 16:0 LPC in report by Okajima *et al*[13]. In our study, however, 18:0 LPC showed almost equipotent activity to 16:0 LPC, although the increase was slightly delayed (Fig 2, 3). Furthermore, 14:0 LPC was less efficacious than 16:0 LPC (Fig 2, 3).

LPC analogues from marine sponge mobilizes Ca^{2+} in HL-60 cells Compounds A-D also mobilized Ca^{2+} in HL-60 cells (Fig 4). Compound C showed the most robust activity in Ca^{2+} mobilization in HL-60 cells (Fig 5). Compound C has an ether linkage instead of an ester bond in the LPC structure. Concentration-response curves for 16:0 LPC and compound C were almost the same, implying that the carbonyl group of LPC is not indispensable for the activity. Compound D, which has a methoxy group at C-2 of the alkyl chain, showed similar potency to compound C, suggesting that the presence of a methoxy group does not modulate the activity of LPC (Fig 5). Compounds A and B increased $[Ca^{2+}]_i$ in HL-60 cells, but were less efficacious than compound C (Fig 5). Furthermore, compound A increased $[Ca^{2+}]_i$ by the smallest magnitude among the tested lipids (Fig 4). Considering the presence of double bonds in both compounds A and B, the reduced efficacy of compound A could be explained by the difference in the ammonium moiety. Except compound A, all of the tested

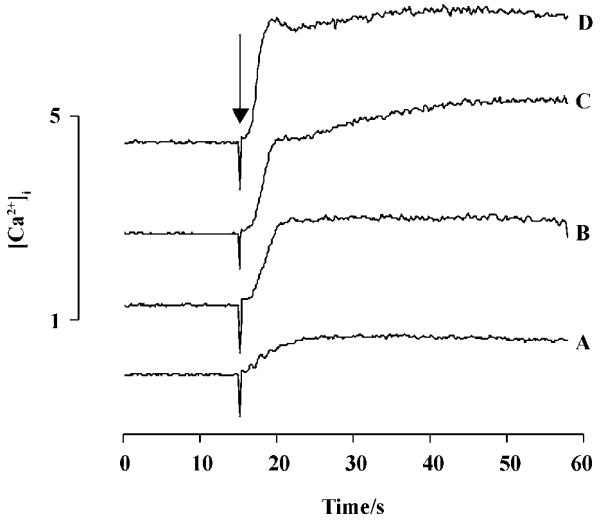

Fig 4. Representative Ca^{2+} traces with 20 μmol/L of compounds A-D.

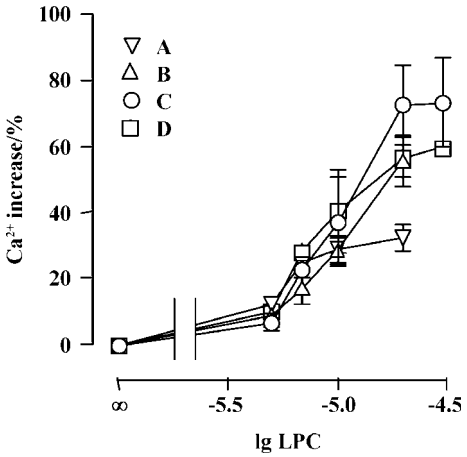

Fig 5. Concentration-response curves of Ca^{2+} mobilization in HL-60 cells. Maximum Ca^{2+} increase by each concentration of compounds A-D was converted to percentage to digitonin-mobilized Ca^{2+} increase. *n*=3-5 independent experiments. Mean±SD.

lipids have a trimethylquaternary ammonium moiety in the choline structure and showed similar activities in Ca^{2+} mobilization (Fig 5). Therefore, the quaternary ammonium part of compound A may be considered as a cause of the reduced Ca^{2+}-mobilizing activity.

Structure-activity relationship of LPC on Ca^{2+} mobilization In the present study, we found that 14:0, 16:0, and 18:0 LPCs increased intracellular Ca^{2+} concentration in HL-60 human leukemia cells and compounds A-D also induced increase of intracellular Ca^{2+} concentration. Comparing concentration-response curves of the tested LPCs and compounds A-D, we could obtain information regarding the structure-activ-

ity relationship of LPC on Ca^{2+} mobilization in HL-60 cells. First, the ester moiety was replaceable with an ether linkage without any influence on the activity. Second, replacement of the choline moiety with an ethanolamine moiety reduced the activity, suggesting that the positively-charged choline structure is important for the increase of intracellular Ca^{2+} concentration. Third, introduction of one or two double bonds did not significantly influence the activity. Fourth, modification of the alkyl chain with a 2-methoxy group also did not significantly reduce the activity. Fifth, change of the chain length affected the activity: lipids with 16 carbons on the alkyl chain induced robust increase of Ca^{2+}. 18:0 LPC showed similar efficacy to 16:0 LPC, although 18:0 LPC-induced Ca^{2+} increase was typically delayed by about 10-15 s to reach the maximum height as compared to the 16:0 LPC-induced increase. Finally, 14:0 LPC was less efficacious than 16:0 LPC.

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