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Identification of lysophosphatidylthreonine with an aromatic fatty acid surrogate as a potent inducer of mast cell degranulation

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ABSTRACT

Upon various stimulations, mast cells (MCs) release a wide variety of chemical mediators stored in their cytoplasmic granules, which then initiates subsequent allergic reactions. Lysophosphatidylserine (LysoPS), a kind of lysophospholipid, potentiates the histamine release from MCs triggered by antigen stimulation. We previously showed through structure-activity studies of LysoPS analogs that LysoPS with a methyl group at the carbon of the serine residue, i.e., lysophosphatidylthreonine (LysoPT), is extremely potent in stimulating the MC degranulation. In this study, as our continuing study to identify more potent LysoPS analogs, we developed LysoPS analogs with fatty acid surrogates. We found that the substitution of oleic acid to an aromatic fatty acid surrogate (C3-pH-*p*-O-C11) in 2-deoxy-1-LysoPS resulted in significant increase in the ability to induce MCs degranulation compared with 2-deoxy-1-LysoPS with oleic acid. Conversion of the serine residue into the threonine residue further increased the activity of MC degranulation both in vitro and in vivo. The resulting super agonist, 2-deoxy-LysoPT with C3-pH-*p*-O-C11, will be a useful tool to elucidate the mechanisms of stimulatory effect of LysoPS on MC degranulation.

1. Introduction

Mast cells (MCs) play a critical role in immediate-type allergic reactions triggered by antigen binding–induced cross-linking of IgE-bound FcεRI (a high-affinity receptor for IgE) and the resulting release of chemical mediators such as histamine and serotonin from their secretary granules, a process known as MC degranulation [1,2]. The released histamine from MCs can cause allergic diseases such as pollinosis, urticaria, atopic dermatitis, and asthma. Identification of factors that modulate MC degranulation would be helpful in providing tools to investigate the molecular mechanisms of allergic reactions as well as to develop anti-allergic drugs.

Lysophosphatidylserine (LysoPS; 1-acyl-2-lyso-PS or 1-lyso-2-acyl-PS) has several biological activities, including a promotion of neurite outgrowth, a suppression of T lymphocyte proliferation and an enhancement of MC degranulation [3]. Among them, the most characterized biological action of LysoPS is its action to MCs. Exogenous phosphatidylserine (PS) or LysoPS strongly enhances the degranulation of rat peritoneal MCs (RPMCs) initiated by FcεRI cross-linking [4,5]. Because LysoPS is ~1000 times more active than PS and LysoPS is readily produced from PS, LysoPS is considered as the true effector for MCs [5]. The action of LysoPS is highly specific; all other lysophospholipids (including lysophosphatidyl-*D*-serine, an optical isomer of lysophosphatidyl-*L*-serine) are reported to be ineffective [6]. Thus, a specific receptor for LysoPS should exist on the plasma membrane of RPMCs.

Four G protein-coupled receptors (GPCRs) (LPS_1/GPR34, LPS_2/P2Y10, LPS_{2L}/A630033H20Rik and LPS_3/GPR174) that were specifically activated by LysoPS have been proposed as candidate MC LysoPS receptors [8,9]. However, they do not appear to be MC LysoPS receptors because none of them reacted with the super agonist, lysophosphatidylthreonine (LysoPT; see below) [10]. In addition, MCs isolated from LPS_1/GPR34-deficient mice were still activated by LysoPS [11]. Thus, the LysoPS receptor on MC has not been identified yet.

For molecular identification of unknown receptor, specific agonists

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with potent activity are definitely useful. We previously synthesized a series of chemically modified LysoPS (*so*-called LysoPS analogs) and tested their ability to promote antigen-induced RPMC degranulation and to activate cloned LysoPS receptors (i.e., LPS_1/GPR34, LPS_2/P2Y10 and LPS_3/GPR174) [10,12,13]. In these studies, LysoPS was regarded as a modular assembly of serine, phosphate, glycerol and fatty acid and modified each module. Indeed, we developed several LysoPS analogs containing simple modifications in individual modules that were found to be potent inducers of MC degranulation, but were incapable of activating the cloned LysoPS receptors. In the first round of our study, modification was focused on the serine and glycerol modules. As a result, a LysoPS analog with an addition of a methyl group at the carbon of the serine residue, i.e., LysoPT, was identified as a potent LysoPS analog in promoting antigen-elicited MC degranulation [10]. In the second round, the modification was focused on the fatty acid module and a number of LysoPS analogs with fatty acid module and aromatic fatty acid modifications were generated [12,13]. Here, a number of these analogs were evaluated for their ability to promote antigen-induced MC degranulation. We identified an aromatic fatty acid surrogate that greatly enhanced the degranulation-promoting activity of LysoPS. By introducing this surrogate into the structure of 2-deoxy-1-LysoPT, we created a super agonist with ~100-fold higher activity than LysoPS.

2. Methods

2.1. Materials

Oleoyl (18:1)-LysoPS was purchased from Avanti Polar Lipids. LysoPS analogs except 2-deoxy-1-C3-pH-*p*-O-C11-LysoPT were synthesized as described previously [10,13], and the chemical structure was confirmed by nuclear magnetic resonance (NMR) and element analysis. We confirmed that the purity of LysoPS analogs was always more than 95%. All rats and mice were purchased from Japan SLC.

2.2. Mast cell degranulation assay in vitro

MCs from the peritoneal cavity of rats and mice were prepared essentially as previously described [10]. The MCs were purified by Percoll (GE Healthcare) in HEPES-buffered Tyrode (HBT) solution (pH 7.4) and suspended at a cell density of 5×10^4/mL (in 0.2 mL) in HBT solution containing 0.01% BSA and stimulated with the indicated dose of each LysoPS analog (nM–μM) in the presence of concanavalin A (Sigma Aldrich) (100 μg/mL for RPMC and 10 μg/mL for mouse peritoneal MCs), which is known to cross-link the FcεRI receptor, at 37 °C. After 15 min, cells were added with cold 0.01% BSA/ HBT solution to stop the degranulation reaction. Then, cell suspensions were centrifuged for 5 min at ×860*g*. The histamine content in the supernatant was determined by the *o*-phthalaldehyde (OPA) fluorometric assay as shown below [10,14]. HCl (1 M) was added to the supernatant for measuring the total histamine. Next NaOH (1 M) was added, followed by OPA reagent. After 4 min, HCl (3 M) was added to stop the OPA reaction. The solution was then transferred to black 96-well flat bottom plate (Greiner) and the fluorescence at 450 nm resulting from excitation at 360 nm was measured using a microplate reader (FlexStation3, Molecular Devices). Histamine release was calculated as a percentage of the total histamine recovered from non-stimulated cells (treated with 0.01% BSA/HBT solution). Values that are subtracted by that of negative control group are shown in Figure. Values for histamine release are presented as the means ± SE for several replicate experiments on different samples of pooled cells, each in triplicate. All animal procedures were done according to the guidelines for care and use of laboratory animals approved by Tohoku University.

2.3. Evaluation of hypothermic effect of LysoPS analogs

LysoPS and LysoPS analogs were dissolved in PBS containing 0.1% bovine serum albumin and were injected intravenously into mice. Rectal temperatures were measured with an electronic thermometer (Physitemp Instruments) every 5 or 10 min for 70 min

2.4. TGFα shedding assay

Transforming growth factor α (TGFα) shedding assay was performed as previously described [9]. Briefly, HEK293A (for LPS_1 and LPS_2) or HEK293FT (for LPS_3) cells were transfected with two kinds of plasmids vectors encoding LysoPS receptors and alkaline phosphatase-tagged TGFα. For LPS_1, a plasmid vector encoding Gαq/i1 was co-transfected [12,15]. After 24 h, cells were harvested and mixed with a test compound for 1 h. Compound-induced AP-TGFα release from cells was evaluated by determining the AP activity in the conditioned media. Values for AP-TGFα release were presented as the means ± SD for three independent experiments, each in triplicate.

2.5. Synthesis of 2-deoxy-1-C3-pH-p-O-C11-LysoPT

2-deoxy-1-C3-pH-*p*-O-C11-LysoPT was synthesized from *L*-threonine, mono-protected propane-1, 3-diol and fatty acid surrogate (C3-pH-*p*-O-C11) in a similar manner as in [13]: mono-protected propane-1,3-diol, i.e., 3-((*tert*-butyldimethylsilyl)oxy)propan-1-ol, synthesized from propane-1,3-diol, was connected with O-phosphanyl N- and C-terminal-diprotected *L*-threonine, tert-butyl O-(tert-butoxy(diisopropylamino)phosphanyl)-N-(*tert*-butoxycarbonyl)-L-threoninate by using phosphoramidite method. After removing the protective group (a *tert*-butyldimethylsilyl group) from the alcohol moiety, the free hydroxyl group was esterified with the fatty acid surrogate (C3-pH-*p*-O-C11), to yield protected. Global deprotection with TFA (trifluoroacetic acid) and purification by silica gel column chromatography furnished the 2-deoxy-1-LysoPT analog. 2-deoxy-1-C3-pH-*p*-O-C11-LysoPT as TFA salt (0.67 TFA) satisfied elemental analysis (Purity: > 99.7%). HPLC purity (area normalization method): 95%.

3. Results and discussion

3.1. LysoPS analogs with an aromatic fatty acid surrogate, C3-pH-p-O-C11, is a potent mast cell degranulation inducer

The structure of LysoPS analogs used in this study is shown in (Fig. 1). Because of the ease of chemical synthesis and the fact that they don't require a *sn*-2 hydroxy group of LysoPS for MC degranulation-inducing activity, most of the LysoPS analogs tested in this study did not have the *sn*-2 hydroxy group (deoxy-LysoPS analogs). We replaced the fatty acid of LysoPS with various aromatic fatty acid surrogates (Fig. 1). The LysoPS analogs tested were 2-deoxy-1-C3-pH-*o*-O-C7-lysophosphatidylserine (LysoPS), 2-deoxy-1-C3-pH-*o*-O-C9-LysoPS, 2-deoxy-1-C3-pH-*o*-O-C11-LysoPS, 2-deoxy-1-C3-pH-*m*-O-C11-LysoPS and 2-deoxy-1-C3-pH-*p*-O-C11-LysoPS. Each LysoPS analog (+ a previously characterized deoxy-1-LysoPS (18:1) and LysoPS (18:1)) was tested for their ability to stimulate concanavalin A-induced MC degranulation from RPMC. The reactivity of each LysoPS analog in stimulating MC degranulation, as judged by histamine release from RPMCs, is shown in Fig. 2. and the results are summarized in Table 1. Modification of the fatty acid module significantly affected the degranulation-stimulating activity of LysoPS. Among the 2-deoxy-1-LysoPS analogs with fatty acid surrogates, 2-deoxy-1-C3-pH-*p*-O-C11-LysoPS showed the highest activity with an EC_{50} value about 40 nM (Fig. 2 and Table 1).