BBA - Molecular and Cell Biology of Lipids 1863 (2018) 1458–1468

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Lysophosphatidylinositols in inflammation and macrophage activation: Altered levels and anti-inflammatory effects

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ARTICLE INFO

Keywords:
Lysophospholipids
Lipid mediator
Phosphatidylinositol
IBD

ABSTRACT

Lysophosphatidylinositols (LPI) are bioactive lipids that are implicated in several pathophysiological processes such as cell proliferation, migration and tumorigenesis and were shown to play a role in obesity and metabolic disorders. Often, these effects of LPI were due to activation of the G protein-coupled receptor GPR55. However, the role of LPI and GPR55 in inflammation and macrophage activation remains unclear. Therefore, we thought to study the effect of macrophage activation and inflammation on LPI levels and metabolism. To do so, we used J774 and BV2 cells in culture activated with lipopolysaccharides (LPS, 100 ng/mL) as well as primary mouse alveolar and peritoneal macrophages. We also quantified LPI levels in the cerebellum, lung, liver, spleen and colon of mice with a systemic inflammation induced by LPS (300 μg/kg) and in the colon of mice with acute colitis induced by dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNBS) and chronic DSS-induced colitis.

Our data show that LPS-induced macrophage activation leads to altered LPI levels in both the cells and culture medium. We also show that cytosolic phospholipase A2α (cPLA2α) and α/β-hydrolase domain 6 (ABHD6) are among the enzymes implicated in LPI metabolism in J774 macrophages. Indeed, ABHD6 and cPLA2α inhibition increased 20:4-LPI levels in LPS-activated macrophages. Furthermore, incubation of LPS-activated cells with LPI decreased J774 activation in a GPR55-dependent manner. In vivo, LPI levels were altered by inflammation in the liver, spleen and colon. These alterations are tissue dependent and could highlight a potential role for LPI in inflammatory processes.

1. Introduction

Lysophospholipids are glycerophospholipids in which the glycerol moiety is substituted by a single acyl chain. They are constituted of a constant “polar” moiety linked to the glycerol backbone (defining the family) and, within each family, of a variable acyl chain, on the sn2 or sn1 position of the glycerol. These lipids, once considered as simple intermediates in the metabolism of phospholipids, are now recognized as bioactive lipids. The most studied lysophospholipid is certainly lysophosphatidic acid (LPA) which has been implicated in cell proliferation and survival, cancer, inflammation, pain, and metabolic syndrome [1,2]. Lysophosphatidylcholines (LPC), the most abundant lysophospholipids, seem to play a role both in the initiation and resolution of inflammation, possibly through a G protein-coupled receptor (GPCR), GPR132 [2,3]. Lysophosphatidylserine (LysoPS) production by activated and apoptotic neutrophils is a signal leading to their phagocytosis by macrophages, thus triggering resolution of inflammation [4]. However, much less is known on the properties of other lysophospholipids such as the lysophosphatidylinositols (LPI) which remain to date the less studied lysophospholipids. Early studies reported their effects on insulin secretion and on the cell cycle [5–8]. More recently, the GPCR GPR55 was put forth as an important molecular target mediating LPI's effects [9–11]. The molecular LPI species were shown to have different agonist potencies towards GPR55 [9]. Indeed, 2-arachidonoyl LPI is the most potent agonist of GPR55, while 1-palmitoyl LPI is a weak partial agonist. Linoleoyl, oleoyl and stearoyl

Abbreviations: LPS, lipopolysaccharides; LPI, lysophosphatidylinositols; LPE, lysophosphatidylethanolamines; LPG, lysophosphatidylglycerols; LysoPS, lysophosphatidylserines; LPC, lysophosphatidylcholines

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https://doi.org/10.1016/j.bbalip.2018.09.003
Received 26 April 2018; Received in revised form 26 August 2018; Accepted 9 September 2018
Available online 11 September 2018
1388-1981/

LPI were less potent agonists than arachidonoyl LPI [9]. Since then, LPI and GPR55 have been associated with several pathophysiological processes [12]. For instance LPI and GPR55 have been implicated in cell proliferation, migration and tumorigenesis [13]. Moreover, the LPI-GPR55 axis clearly plays a role in obesity and metabolic disorders [14]. However, the role of LPI and GPR55 in inflammation and immune cell activation remains unclear.

Complicating the study of the effects of LPI is the intricacy of their metabolic pathway. Phosphatidylinositols (PI) are the key precursors of LPI. Upon hydrolysis by a phospholipase A1 (PLA1) or a phospholipase A2 (PLA2), PI will generate 2-acyl LPI and 1-acyl LPI, respectively (Suppl. Fig. S1). Among the PLA1 enzymes, DDHD1 (also known as PA-PLA1) was described for its ability to produce 2-arachidonoyl LPI in vitro and in vivo [15]. PLA2 enzymes are expected to generate LPI, however PLA2 isoforms have been mostly studied in regards to arachidonic acid and eicosanoid synthesis. Among these, cPLA2α, which has a preference for releasing arachidonic acid from the sn-2 position of phospholipids, is implicated in the biosynthesis of LPI [16–18]. However, while activation of cPLA2α and the release of arachidonic acid from membrane phospholipids constitute key features of inflammation, little is known on the effect of inflammation on the cell and tissue levels of LPI.

Following their synthesis, LPI can be reacylated to PI by acyltransferases [19,20] or deacylated by lysophospholipases. LPI-specific PLA and PLC activities have been reported although the molecular characterization of these specific lysoPLA and lysoPLC enzymes is still lacking [19]. Of note, cPLA2α has been reported as capable of deacylating LPI into glycerophosphoinositol [21], therefore it could be implicated both in the synthesis and catabolism of LPI. More recently, α/β hydrolase domain 6 (ABHD6), a monoacylglycerol hydrolase, was also implicated in lysophospholipid hydrolysis, although this was not directly shown for LPI [22]. Finally, autotaxin, a lysoPLD responsible for LPA biosynthesis from LPC, is also able to convert LPI to LPA although less efficiently than LPC [23].

Considering (i) the complexity of the metabolic pathways of LPI, (ii) the fact that several enzymatic activities of the pathway have not been characterized and (iii) that inflammation could affect several enzymes of these pathways, we decided to study the interplay between LPI and inflammation. Thus, we used an in vitro approach to study the consequences of macrophage activation on LPI and other lysophospholipid levels. We then show, in vivo, that inflammation alters the levels of LPI in numerous tissues. As LPI levels are altered upon inflammation and LPS-induced macrophage activation, we also wondered whether LPI could modulate J774 cell activation. Thus, this report points to LPI as interesting bioactive lipids in the context of inflammation.

2. Materials and methods

2.1. Materials

Bovine liver LPI (major specie 18:0 LPI), bovine brain LysoPS (major specie 18:0 LysoPS), egg LPE (major specie 18:0 LPE), egg LPC (major species 16:0 and 18:0 LPC), 18:0 LPG and 18:0 LPA as well as internal standards were purchased from Avanti polar lipids. WWL70 was purchased from Cayman Chemicals. HEPES, sucrose, EGTA, glycerol, $CaCl_2$, PI from bovine liver, dipalmitoylglycerol (DAG), arachidonoyltrifluoromethylketone (ATFMK), bromoenol lactone (E-6-(Bromoethylene) tetrahydro-3-(1-naphthyl)-2H-pyran-2-one, BEL) and CID16020046 (4-[4,6-Dihydro-4-(3-hydroxyphenyl)-3-(4-methylphenyl)-6-oxopyrrolo [3,4-*c*]pyrazol-5(1H)-yl]-benzoic acid) were all bought from Sigma Aldrich. Lipopolysaccharides (LPS) from *E. coli* (O55:B5) and fetal bovine serum were also purchased from Sigma Aldrich. Dextran sulfate sodium (DSS) was purchased from TdB Consultancy. pDream2.1 vector containing the human GPR55 coding sequence was obtained from GenScript corporation. Lipofectamine LTX plus was purchased from Life Technologies. Cell culture media, trypsin and penicillin-streptomycin were purchased from Gibco.

2.2. Animal models

C57BL/6 J mice (8–10 weeks of age) were purchased from Charles River Laboratories (Brussels, Belgium) and were housed under standard conditions (12 hours light/dark cycle, and controlled 22 °C temperature) and supplied with water and food ad libitum. All animal care and experimental procedures were conducted in accordance with the guidelines of the local ethics committee and in accordance with European directive 2010/63/EU, which was transformed into the Belgian Law of May 29, 2013 regarding the protection of laboratory animals. All experimental protocols were approved by the Université catholique de Louvain animal ethics committee (study agreement 2014/UCL/MD/001, laboratory agreement LA1230314).

For LPS-induced inflammation in mice, LPS (300 μg/kg, in saline containing 0.1% Tween 80) or vehicle were administered i.p and mice (7 mice/group) were sacrificed by cervical dislocation 4 or 8 h later [24,25].

Three models of colitis were used.

TNBS-induced colitis: Mice (10 mice/group) were food-deprived for 24 h before administration of trinitrobenzene sulfonic acid (TNBS). Mice were anesthetized by intraperitoneal (i.p.) injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). TNBS (100 mg/kg, in 50 μL 0.9% NaCl-ethanol, 50:50, v/v) was administered intrarectally into the colon (4 cm from the anus) using a cannula. Control mice received 50 μL of a 0.9% NaCl-ethanol (50:50, v/v) solution. To ensure a homogeneous distribution and retention of TNBS (or vehicle) within the colon, mice were held by the tail, in a vertical position, for 60 s after administration [26].

DSS-induced colitis:

Acute colitis: Colitis was induced by adding dextran sulfate sodium (DSS, 5%) to the drinking water of mice for 5 days. Mice (8 per group) were sacrificed once the colitis was well established i.e. at day 7 [26,27].

Chronic colitis: Chronic colitis was induced by following 3 cycles consisting in 7 days of DSS (2.5%) in the drinking water followed by 3 weeks of normal drinking water [28]. Mice (8 per group) were sacrificed during the recovery phase after the third cycle of DSS.

For all models, body weight and food and water intake were monitored daily for the duration of the study. Following sacrifice of the mice, colons were quickly recovered and snap-frozen in liquid nitrogen before storage at −80 °C until their analysis.

2.3. Cell culture

The murine macrophage cell line J774 (a generous gift from M.-P. Mingeot-Leclercq, LDRI, UCL, Belgium and from J. Leclercq, LDRI, UCL, Belgium) was cultured under standard conditions (37 °C in a humidified 5% CO2 incubator) in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and antibiotics. BV2 microglial-like cells (a generous gift from E. Hermans, IONS, UCL, Belgium) were grown in high-glucose DMEM medium with 10% FBS and antibiotics.

Cells were seeded overnight into 24-well plates (2.5×10^5 cells per well) for qRT-PCR experiments, and into 35 mm dishes (10×10^6 cells per dish) for lipid quantification, and the experiments performed the next morning. Cells were then incubated with fresh culture medium containing 100 ng/mL of LPS for 8 h (based on previous experiments [24,28]). For all experiments, a control condition was performed, where cells were seeded concomitantly but were only incubated with vehicle (DMSO, 0.05%) in the absence of LPS. When the cells were treated, the compounds of interest were added 1 h prior to LPS.