European Journal of Pharmacology 825 (2018) 63–74

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

Ursodeoxycholyl lysophosphatidylethanolamide negatively regulates TLR-mediated lipopolysaccharide response in human THP-1-derived macrophages

Alzbeta Horvatova[a,b], Tanyarath Utaipan[c], Ann-Christin Otto[a], Yuling Zhang[a], Hongying Gan-Schreier[a], Petr Pavek[b], Anita Pathil[a], Wolfgang Stremmel[a], Walee Chamulitrat[a,*]

[a] Department of Internal Medicine IV, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University in Prague, Heyorovskeho 1203, 500 05 Hradec Kralove, Czech Republic
[c] Department of Pre-Clinic, Faculty of Sciences and Technology, Prince of Songkla University, Pattani Campus, 94000 Pattani, Thailand

ARTICLE INFO

Keywords:
Bile-acid and phospholipid conjugates
Cytokines
Macrophages
Lipopolysaccharides
Signaling pathways
Lipid rafts

ABSTRACT

The bile acid-phospholipid conjugate ursodeoxycholyl oleoyl-lysophophatidylethanolamide (UDCA-18:1LPE) is an anti-inflammatory and anti-fibrotic agent as previously shown in cultured hepatocytes and hepatic stellate cells as well as in in vivo models of liver injury. We hypothesize that UDCA-18:1LPE may directly inhibit the activation of immune cells. We found that UDCA-18:1LPE was capable of inhibiting the migration of phorbol ester-differentiated human THP-1 cells. We examined anti-inflammatory activity of UDCA-18:1LPE during activation of THP1-derived macrophages. Treatment of these macrophages by bacterial lipopolysaccharide (LPS) for 24 h induced the release of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β. This release was markedly inhibited by pretreatment with UDCA-18:1LPE by ~ 65–90%. Derivatives with a different fatty-acid chain in LPE moiety also exhibited anti-inflammatory property. Western blotting and indirect immunofluorescence analyses revealed that UDCA-18:1LPE attenuated the expression of phosphorylated p38, MKK4/MKK7, JNK1/2, and c-Jun as well as nuclear translocation of NF-κB by ~ 22–86%. After LPS stimulation, the Toll-like receptor adaptor proteins, myeloid differentiation factor 88 and TNF receptor associated factor 6, were recruited into lipid rafts and UDCA-18:1LPE inhibited this recruitment by 22% and 58%, respectively. Moreover, LPS treatment caused a decrease of the known cytoprotective lysophosphatidylcholine species containing polyunsaturated fatty acids by 43%, and UDCA-18:1LPE co-treatment reversed this decrease. In conclusion, UDCA-18:1LPE and derivatives inhibited LPS inflammatory response by interfering with Toll-like receptor signaling in lipid rafts leading to an inhibition of MAPK and NF-κB activation. These conjugates may represent a class of lead compounds for development of anti-inflammatory drugs.

1. Introduction

Sustained homeostatic alteration with simultaneous activation of inflammatory pathways are central events occurring in many chronic diseases, such as liver disease, obesity, autoimmune disorders, metabolic syndromes, atherosclerosis, and cancer (Hansson et al., 2002; Sun and Karin, 2012). The prevalence of chronic diseases is increasing thus constituting public health problems worldwide and cost escalation for the development of anti-inflammatory drugs (DiMasi et al., 2003). Among these drugs, biologically active lipids, such as polyunsaturated fatty acids (PUFAs) (Lordan et al., 2011; Simopoulos, 2004), sterols (Bouic, 2002; Mencarelli et al., 2009) and phospholipids (PL) (Küllenberg et al., 2012; Stremmel and Gauss, 2013), also represent anti-inflammatory agents by targeting multiple tissues (Crielaard et al., 2012). With lipid-based design strategy, we developed a specific drug-targeting technology by a conjugation between a PL and a sterol, and for the latter a protective bile acid was chosen. Our first synthesized conjugate was ursodeoxycholyl oleoyl-lysophosphatidylethanolamide (UDCA-18:1LPE) (Chamulitrat et al., 2009). While UDCA-LPE inhibits apoptosis in hepatocytes (Chamulitrat et al., 2009; Pathil et al., 2011) and biliary epithelial cells (Sellinger et al., 2015), it also inhibits inflammatory response in Kupffer cells (Pathil et al., 2011), and fibrogenic activation in hepatic stellate cells (Pathil et al., 2014). Thus, the protective effect of UDCA-LPE observed in many cellular systems renders it appropriate for treatment of inflammatory diseases as previously shown in fulminant hepatitis (Pathil et al., 2011; Utaipan et al., 2017),

* Correspondence to: Department of Internal Medicine IV, University of Heidelberg Hospital, Im Neuenheimer Feld 345, EG, 69120 Heidelberg, Germany.
E-mail address: Walee.Chamulitrat@med.uni-heidelberg.de (W. Chamulitrat).

https://doi.org/10.1016/j.ejphar.2018.02.030
Received 22 November 2017; Received in revised form 15 February 2018; Accepted 19 February 2018
Available online 20 February 2018
0014-2999/

non-alcoholic steatohepatitis (Pathil et al., 2012, 2014, 2015), and ischemia reperfusion (Wang et al., 2015) in vivo models.

It is noted that UDCA-18:1LPE exhibits protection as an intact conjugate whereby the parental UDCA and 18:1LPE do not show protection as shown in in vitro (Chamulitrat et al., 2009) and in vivo (Pathil et al., 2015) experiments. This provides a hint that the hydrophobicity of the conjugate may be crucial in rendering its ability to interact with or accumulate into membranes (Fahey et al., 1995; Esteves et al., 2015). This may be the molecular basis for UDCA-18:1LPE as a drug candidate for membrane lipid therapy (Escribá et al., 2015), particularly in the membranes of macrophages. Here, we aimed to test the efficacies of UDCA-LPE in lipopolysaccharide (LPS)-activated human THP-1-derived macrophages and determined the underlying mechanisms involved in the modulation of inflammatory response via receptor signaling (Vallabhapurapu and Karin, 2009) and lipid-raft membranes (Simons and Toomre, 2000). As UDCA-18:1LPE is able to modulate hepatocellular PL (Chamulitrat et al., 2012, 2013; Pathil et al., 2015), the effects of UDCA-18:1LPE on PL profiles of activated macrophages were also determined.

2. Materials and methods

2.1. Chemicals

All PL standards, 14:0 LPE, 16:0 LPE, and 18:1 LPE were obtained from Avanti Lipids, Inc, (Alabaster, AL, USA). IFN-γ was obtained from Immuno Tools GmbH, Friesoythe, Germany. L-α-lysophosphatidylcholine (LPC) type V from bovine brain, LPS from *E. Coli* 055:B5, and all other chemicals were obtained from Sigma Aldrich (Taufkirchen, Germany) unless stated otherwise.

2.2. Synthesis of UDCA-LPE and derivatives

The synthesis of UDCA-18:1LPE was performed by ChemCon (Freiburg, Germany) using the published procedures (Chamulitrat et al., 2009). Custom syntheses of UCDA-PE (UDCA-18:1,18:1PE), UDCA-14:0LPE, UDCA-16:0LPE, UDCA-18:0LPE, UDCA-18:3LPE, UDCA-20:4LPE, and chenodeoxycholic acid (CDCA) − 18:1LPE were performed by Med Chem 101 (Conshohocken, PA, USA).

2.3. Cell culture

THP-1 human monocytic cell line (Cell Line Service GmbH, Eppelheim, Germany) was kindly provided by Dr. W. Chunglok of Walailak University, Thailand. Cells were cultivated in suspension in RPMI-1640 medium (Gibco®, Darmstadt, Germany), supplemented with 10% heat-inactivated FBS (Gibco®), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C, 5% CO_2. THP-1 cells were differentiated into macrophages by treatment with 100 nM phorbol 12-myristate 13-acetate (PMA) for 72 h. Differentiated cells were pretreated with UDCA-LPE for 1 h followed by treatment with 100 ng/ml LPS with or without 5 pg/ml IFN-γ. Treated cells were harvested 24 h later.

2.4. Cytotoxicity assay

Cell viability in THP-1-derived macrophages was determined by 3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyltetrazolium bromide (MTT) assay. After treatment, differentiated THP-1 cells (1×10^5 cells/well in 24-well plates) were incubated overnight in fresh medium and subsequently with 0.2 mg/ml MTT for 4 h at 37 °C. The solution was discarded and formazan crystals were solubilized in 500 µl DMSO per well. The absorbance of the formazan solution was measured using a microplate reader (Thermo Fisher, USA) at 560 nm and 670 nm (for background subtraction). Cell viability was calculated according to: Cell viability (%) = [(Abs treated sample/ Abs untreated sample) × 100].

2.5. Enzyme linked immunoassays

Differentiated THP-1 cells (1×10^5 cells/well) seeded in a 24-well plate were pretreated with UDCA-18:1LPE or derivatives and subsequently treated with 100 ng/ml LPS for 24 h. Proinflammatory cytokines, TNF-α, IL-6, and IL-1β in cultured medium were quantified by using enzyme-linked immunosorbent (ELISA) kits obtained from BioLegend (Cambridge, UK).

2.6. Cell migration assay

Migration of THP-1-derived macrophages was determined by a wound-closure assay (Liang et al., 2007). Briefly, THP-1 cells (1×10^5 cells/well) seeded in a 24-well plate were differentiated with 100 nM PMA for 72 h. Confluent cells were washed twice with PBS and scratched by a sterile pipette tip to create a wound. Cells were subsequently treated with UDCA-18:1LPE or a negative control tauro-UDCA at 50 µM. Cells were photographed at 4 h, 20 h, and 40 h after treatment. Cropped pictures of the scratched areas were obtained by using Photoshop and were processed for cell counts by using particle analysis of Image J. Number of cells in scratched areas of each treatment group and time point was obtained from 3 independent experiments.

2.7. NF-κB nuclear translocation assay

NF-κB nuclear translocation was studied by indirect immunofluorescence. Cells with seeding density 1.5×10^5 cells/ml were differentiated on 10-mm coverslips coated with gelatin. After treatment, cells were fixed in ice-cold methanol. To avoid unspecific binding of antibody, coverslips were treated with 1% BSA in PBS containing 0.01% Tween-20 at RT for 10 min. Cells were incubated with 1:200 anti-p65 NF-κB primary antibody (#8242, Cell Signaling, Frankfurt, Germany) at RT for 1 h. After washing, cells were incubated with AlexaFluor 488 conjugated anti-rabbit IgG (A11034, Life Technologies) at RT for 1 h followed by nuclear staining with 4′ 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for 10 min. After washing, coverslips were mounted with Entellan™ (Merck, Darmstadt, Germany). Images were taken by an Olympus AX70 microscope (Olympus, Hamburg, Germany). NF-κB nuclear positive cells were counted from ten random fields with a total of 200–300 cells per cover slip. In each experiment, quadruplicate cover slips were stained and counted, and 6 independent experiments were carried out.

2.8. Western blotting

Differentiated THP-1 cells (4.8×10^5 cells/well) seeded in a 6-well plate were pretreated with 50 or 75 µM of UDCA-LPE for 1 h followed by the treatment with 100 ng/ml LPS for 30 min. Cells were lysed, and protein concentrations were determined using Bio-Rad protein^{DC} kit (Bio-Rad, Munich, Germany). Proteins were separated by SDS-PAGE and transferred onto PVDF membranes. Blots were probed with a primary antibody against phospho-ERK1/2 (#9106), phospho-JNK1/2 (#4668), phospho-p38 (#9212), phospho-MKK-7 (#4171), phospho-MKK-4 (#9151), phospho-c-Jun (#9261), phospho-ATF-2 (#9221), MyD88 (#6483) or GAPDH (#2118) obtained from Cell Signaling (Frankfurt, Germany). Antibodies against calnexin (sc-6465) and TRAF-6 (sc-7221) were obtained from Santa Cruz Biotechnology (Heidelberg, Germany), and flotilin-2 (610383) from Becton Dickinson (Heidelberg, Germany). Washed blots were treated with a secondary antibody. Proteins were visualized using Luminata Forte ECL (Millipore, Darmstadt, Germany). Image J was used to quantify the density of protein bands of the targets and loading control GAPDH, and the ratio of protein target/GADPH was calculated.