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Research paper

Progesterone and a phospholipase inhibitor increase the endosomal bis(monoacylglycero)phosphate content and block HIV viral particle intercellular transmission

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ABSTRACT

Progesterone, the cationic amphiphile U18666A and a phospholipase inhibitor (Methyl Arachidonyl Fluoro Phosphonate, MAFP) inhibited by 70%–90% HIV production in viral reservoir cells, *i.e.* human THP-1 monocytes and monocyte-derived macrophages (MDM). These compounds triggered an inhibition of fluid phase endocytosis (macropinocytosis) and modified cellular lipid homeostasis since endosomes accumulated filipin-stained sterols and Bis(Monoacylglycero)Phosphate (BMP). BMP was quantified using a new cytometry procedure and was increased by 1.25 times with MAFP, 1.7 times with U18666A and 2.5 times with progesterone. MAFP but not progesterone or U18666A inhibited the hydrolysis of BMP by the Pancreatic Lipase Related Protein 2 (PLRP2) as shown by *in-vitro* experiments. The possible role of sterol transporters in steroid-mediated BMP increase is discussed.

Electron microscopy showed the accumulation of viral particles either into large intracellular viral-containing compartments or outside the cells, indicating that endosomal accumulation of BMP could block intracellular biogenesis of viral particles while inhibition of macropinocytosis would prevent viral particle uptake.

This is the first report linking BMP metabolism with a natural steroid such as progesterone or with involvement of a phospholipase A1 activity. BMP cellular content could be used as a biomarker for efficient anti-viral drugs.

1. Introduction

The lipid molecules of the viral HIV particle originate from the host cell. Thus altering the host cell lipid metabolism appears as a strategy to fight infectious diseases such as viral infections. It is known that progesterone considerably modifies lipid homeostasis involving cholesterol distribution and metabolism [1,2]. In addition progesterone at high concentrations such as observed in placenta, *i.e.* in the micromolar range, has been reported to inhibit HIV production in placental cells in a non-transcriptional manner [3].

Abbreviations: MAFP, methyl arachidonyl fluoro phosphonate; PROG, progesterone; U18, U18666A; BMP, bis(monoacylglycero)phosphate; LBPA, lyso-bisphosphatidic acid; BDP, bis(diacylglycero)phosphate; BGP, bis(glycero)phosphate; MVB, multivesicular body (late endosome); AZT, zidovudine; LPV, lopinavir.

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Bis(monoacylglycero)phosphate (BMP), a polyglycerophospholipid previously named LysoBisPhosphatidic Acid (LBPA) is linked to cholesterol accumulation in the late endosome compartment [4,5].

In the present study the effects of progesterone on lipid homeostasis and HIV infection have been considered in comparison to cell treatment with another sterol compound, U18666A (3-β-[2-(diethylamino)ethoxy]androst-5-en-17-one), a cationic amphiphile known to trigger cholesterol accumulation in endosomes and the accumulation of the HIV envelope protein Gag in MVBs [6,7]. We also studied the effect of Methyl Arachidonyl Fluoro Phosphonate (MAFP), an analog of arachidonic acid widely used as a cPLA2 inhibitor, since a MAFP-sensitive PLA2 was reported to be involved in the biosynthesis pathway of BMP [8]. Additionally we had earlier reported that MAFP inhibited the infectivity of another enveloped virus, the cytomegalovirus [9].

One of the early events in virus entry is the formation of large plasma membrane ruffles which close into macropinosomes through a process termed macropinocytosis. This process leads to the engulfment of medium peripheral to the cells. Various viruses appear to operate via this process for their entry into cells [10]. The cholesterol content of the plasma membrane plays a key role in macropinocytosis [11]. Thereafter, viruses enter the endosome pathway, fuse with the endosome membrane and can release their replication machinery into the cytosol. HIV biogenesis takes place partly in late endosomes, also called Multivesicular Bodies, where intraluminal vesicles (ILV) also called exosomes are formed. About 10% of the proteins from the endosome are present in HIV viral particles [12]. In HIV-infected cells both HIV viral particles [13] and exosomes are released [14]. It has been proposed that the "exosome pathway" is partly hijacked by the virus [14,15].

BMP is particularly enriched in endosomes [16,17] where it has been shown to play a key role in endosome biogenesis [18,19] and might also be involved in HIV biogenesis prior to its release out of the cell. BMP, previously incorrectly named LBPA (LysoBisPhosphatidic Acid), has also been proposed to favor the fusion of endocytosed viruses such as VSV (Vesicular Stomatitis Virus) with the membrane of late endosomes and to allow the release of their replication machinery in the cytosol of infected cells [18,20].

BMP binds to a protein called Alix [18] that belongs to the endosomal sorting machinery participating in exosome biogenesis [21,22] and Alix also interacts with the HIV envelope protein Gag during viral particle budding [22]. Recently BMP has been shown to regulate infection in endothelial cells by the dengue virus [23]. Incubation of cells with anti-BMP antibody leads to BMP accumulation [24,25], and treatment of endothelial cells with anti-BMP antibody prevents replication of the dengue virus [23]. Therefore increasing cell BMP levels might prevent viral infection.

BMP metabolism is not fully understood. It is biosynthesized from phosphatidyl glycerol (PG) which is hydrolyzed into lyso-PG by a phospholipase A2 (PLA2), and then acylated on the second glycerol moiety by a transacylase [26,27], following which it is supposed to undergo a stereoconfiguration rearrangement [28]. However none of the enzymes involved in this pathway have been characterized at the molecular level. Regarding BMP hydrolysis, it has been initially reported that a phospholipase A2/lipase complex was involved [29], but we recently provided evidence that the recombinant Pancreatic Lipase-Related Protein 2 (PLRP2) was able to hydrolyze monomolecular films of BMP via its phospholipase A1 activity [30].

Experiments were carried out on two cell types established as HIV reservoirs because they are able to retain HIV viral particles inside their endocytic pathway even during therapeutic treatment in patients [31–33]. In patients, infected monocytes play a critical role in disseminating HIV-1 because they retain their ability to differentiate into dendritic cells or macrophages once in the tissues [31]. Cell–cell transmission of the virus accounts for the maintenance of a basal infection in patients treated with Highly Active Anti retroviral viral Therapy (HAART) [34,35]. Therefore we have investigated the effect of progesterone, U18666A, and MAFP on HIV infection propagated by co-cultures between uninfected and infected cells. We used the human monocytic THP-1 cell line in which HIV propagation has been described [36] and BMP biosynthesis pathway is documented [26]. We also detected the Pancreatic Lipase Related Protein 2 (PLRP2) in this cell line [30]. In the case of macrophages, which store HIV for longer periods than monocytes, we used human monocyte-derived macrophages (MDM).

We devised a method to quantify BMP directly in cells by flow cytometry using an anti-BMP antibody. This enabled us to show that progesterone triggered an increase in the BMP content greater than with U18666A or MAFP treatment. The three compounds also inhibited macropinocytosis as monitored by dextran uptake. Progesterone was the most efficient inhibitor of HIV infection in human monocyte-derived macrophages compared with U18666A and MAFP.

The mechanism by which the three compounds increased the BMP content was considered with respect to the PLRP2 activity. We showed that MAFP inhibited the phospholipase A1 activity of recombinant PLRP2, whereas the steroid compounds progesterone and U18666A had no effect on this enzyme, suggesting another mode of regulation of BMP metabolism. A putative role of the endosomal sterol transporters such as NPC1 in BMP accumulation is discussed.

2. Material and methods

2.1. Materials

Progesterone and filipin were supplied by Sigma–Aldrich (Lyon, France). MAFP and U18666A were obtained from Santa Cruz Biotechnology, Santa Cruz, California.

Bis(monoacylglycero)phosphate [18:1 BMP (*S*,*R* isomer) (ammonium salt)] was from Avanti Polar Lipids (COGER, France). Thin layer chromatography plates and solvents for lipid separation were supplied by Merck (France).

Lopinavir and zidovudine (AZT) were obtained from the NIH Research Reagents Program, Germantown, MD, USA. Culture media and FITC-dextran (MW 10,000) were supplied by Invitrogen SARL, (Cergy Pontoise, France). Macrophage colony stimulating factor (M-CSF) was purchased from Miltenyi Biotec SAS (Paris, France). QIAamp Viral mini kits and QIAamp DNA blood mini kits were purchased from Qiagen SA (Courtaboeuf, France). RT-PCR kits were from Roche Diagnostics, Meylan, France. Pacific blue anti-CD4, APC-anti-CD54 (ICAM-1), FITC anti-CD11a (LFA-1), PE-anti-184 (CXCR4) and PerCP-Cy5.5 anti-CD195 (CCR5) and compensation beads were obtained from BD Biosciences, Le Pont de Claix, France. PE-labeled goat anti-mouse IgG antibody was from AbDSerotec, Colmar, France. Anti-HIV-1 antibody (KC-57 FITC) was from Beckman Coulter, Fullerton, USA.

Anti-LBPA(BMP) 6C4 antibody was a generous gift from T. Kobayashi (Lipid Biology Laboratory, RIKEN Institute, Saitama, Japan).

Recombinant human pancreatic lipase-related protein 2 (HPLRP2) was produced in yeast and purified as previously described [37].

2.2. Cell culture

Human promonocytic THP-1 cells were purchased from the ATCC (ATCC number TIB-202™) and maintained at $5 \cdot 10^5$ cells/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (10% FCS RPMI).