Virus Research 214 (2016) 71–79

Contents lists available at ScienceDirect

Virus Research

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

Inhibition of herpes virus infection in oligodendrocyte cultured cells by valproic acid

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ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form 17 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
HSV-1
Valproic acid
Oligodendrocytes

ABSTRACT

Valproic acid (VPA) is a small fatty acid used for treatment of different neurologic diseases such as epilepsy, migraines or bipolar disorders. VPA modulates different processes of cell metabolism that can lead to alterations in susceptibility of several cell types to the infection of *Human Immunodeficiency Virus* (HIV), *Epstein-Barr virus (EBV)*, as well as to exert an inhibitory effect on the replication of different enveloped viruses in cultured cells. Taken these data into account and the fact that HSV-1 has been involved in some neuropathies, we have characterized the effect of VPA on this herpesvirus infection of the differentiation/maturation-inducible human oligodendrocyte cell line HOG, which resulted more susceptible to VPA inhibition of virus growth after cell differentiation. In these cells, the role of VPA in virus entry was tackled. Incubation with VPA induced a slight but reproducible inhibition in the virus particles uptake mainly observed when the drug was added in the adsorption or early upon infection. In addition, transcription and expression of viral proteins were significantly downregulated in the presence of VPA. Remarkably, when the infective viral production was assessed, VPA dramatically blocked the detection of infectious HSV-1 particles. Herein, our results indicate that VPA treatment of HOG cells significantly reduces the effect of HSV-1 infection, virus entry and productivity without affecting cellular viability.

1. Introduction

Valproic acid, VPA (2-propylpentanoic acid, VPA) is a branched short-chain fatty acid that affects different metabolic targets. VPA inhibits histone deacetylases (HDACs) in the cell nucleus, modulates glutamatergic and γ-aminobutyric (GABA) pathways and Na^+ channel in cellular membranes, as well as glycogen synthase kinase 3 (GSK3) and protein kinase A (PKA) and alters lipid metabolism (Countryman et al., 2008; Terbach and Williams, 2009). From a pharmacological point of view, VPA is a compound with clinical anticonvulsant and mood-stabilizing effect currently used for treatment of neurological disorders such as bipolar or epilepsy (Bruni and Wilder, 1979; Li et al., 2015; Tanamachi et al., 2015; Terbach and Williams, 2009). The increase in GABA neurotransmission is involved in its mechanism of action (Bialer, 2012; Nanau and Neuman, 2013). Compared to phenobarbitone barbiturate prescribed for seizures and sleep disorders, intravenous VPA showed a better safety profile (Brigo et al., 2013). Furthermore, the first generation of antiepileptic drugs collectively termed "antiepileptics", such as VPA (liquid at room temperature), sodium valproate (solid salt) or a combination of the two (valproate semisodium), have been successfully used for preventing migraine attacks, reducing drastically the frequency of headaches (Linde et al., 2013). However, some shadows regarding the clinical use of VPA require further assessment. Hepatic, mitochondrial, neurological or metabolic adverse drug reactions have been reported after monotherapy or polytherapy VPA prescription (Nanau and Neuman, 2013) and the incidence of VPA-associated pancreatitis has been estimated to be 1:40,000 (Yaman et al., 2013).

Along the course of the last years, VPA has emerged as a potential antiviral or even antitumor agent (Vazquez-Calvo et al., 2013; Vazquez-Calvo et al., 2011; White and Frampton, 2013). As an inhibitor of the histone deacetylase (HDACi), VPA has been used in a combined treatment with the lytic equine herpesvirus type 1 (EHV-1) against two human glioblastoma cell lines (White and Frampton, 2013). Interestingly, VPA increased virus entry, replication, cell

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http://dx.doi.org/10.1016/j.virusres.2016.01.009
0168-1702/

to cell spread and lysis, improving the oncolytic effect of EHV-1. Furthermore, VPA was able to reactivate latent Kaposi's sarcoma-associated herpesvirus (KSHV), significantly remodeling the viral genome topology and the chromatin architecture of infected primary effusion lymphoma cells (Shin et al., 2014). On the other hand, the inhibitory effect of this compound on the multiplication of different enveloped viruses has been reported (Vazquez-Calvo et al., 2013; Vazquez-Calvo et al., 2011).

VPA is also a well-known glycogen synthase kinase 3 beta (GSK3β) inhibitor. In this regard, it has been published how this fatty acid inhibits the release of soluble CD154 induced by efavirenz (EFV) in human immunodeficiency virus (HIV)-infected individuals. EFV, a non-nucleoside reverse transcriptase inhibitor, increases the release of circulating CD154 after HIV infection and activates GSK3β in platelets, the main source of this kind of CD154 (Davidson et al., 2013). It is worth noting that CD154 can be elevated in the plasma and cerebrospinal fluid of HIV infected-cognitively impaired patients, contributing to the blood brain barrier (BBB) permeability *in vivo* (Davidson et al., 2013). In this model, VPA would act as an antiplatelet activity drug and neuroprotective after HIV infection (Davidson et al., 2011; Schifitto et al., 2006). The improvement of BBB integrity by VPA has been observed in other systems as well (Dash et al., 2010).

Taking into consideration the fact that VPA interferes with cellular lipid metabolism, its effect as antiviral agent was ascertained in several viral families, including both human and animal health relevant viruses, such as flavivirus, arenavirus, togavirus, picornavirus, poxvirus or rhabdovirus (Vazquez-Calvo et al., 2011). VPA was very efficient inhibiting multiplication of enveloped viruses, while naked viruses remained unaltered. In fact, the interference with the viral cycle might happened at different steps of enveloped virus infection either causing the blockage of viral protein synthesis and RNA replication as in the case of West-Nile virus infection, or rather affecting cell release and infectivity of the viral particles produced in cells infected with vesicular stomatitis virus, (Vazquez-Calvo et al., 2013).

Several virus families have been involved in neuropathology and demyelinating disease (Bello-Morales et al., 2014; Bello-Morales et al., 2005; Fazakerley and Walker, 2003; Tsunoda and Fujinami, 2002). In particular and relating to multiple sclerosis (MS), the oligodendrocytes (OLs), the myelin-forming cells in the central nervous system (CNS), may be the initial target for the pathogenic onset (Bello-Morales et al., 2014; Ji et al., 2010; Kakalacheva et al., 2011; Swanborg et al., 2003). In this respect, members of the viral family *Herpesviridae* are been studied/identified as feasible infectious agents involved in the etiology and/or development of this autoimmune disease (Alvarez-Lafuente et al., 2008; Bello-Morales et al., 2014; Christensen, 2005; Ji et al., 2010; Simmons, 2001; Sotelo, 2007; Swanborg et al., 2003). Our group has focused on the study of herpes simplex type 1 (HSV-1), a virus linked to both neurodegenerative and demyelinating diseases (Pietropaolo et al., 2005; Rizzo et al., 2012; Ruprecht et al., 2006; Sanders et al., 1996). Previously, it was described the synergism observed between alpha-2-macroblobulin (A2M), one of the major proteins of plasma that has been implicated in chronic neurodegenerative processes (Dow et al., 1999; Kruger et al., 2000; Rudrasingham et al., 1999) and HSV-1 during the infection of neuronal cells (Alonso et al., 2001). A2M seemed to interact directly with HSV-1 and modulate the course of the infection in SKNMC human neuroblastoma cells (Alonso et al., 2001). On the other hand, the human precursor oligodendroglial KG-1C cell line exhibited high susceptibility to HSV-1 infection with notable progression of the cytopathic effect with a significant increase of MAL2, a subpopulation of detergent-insoluble lipid raft protein (Bello-Morales et al., 2005). Moreover, the HOG cell line derived from a human oligodendroglioma, as well as primary oligodendrocyte precursor cells (OPCs), both susceptible to induced to differentiate into a mature stage, were capable of being infected by HSV-1 in a differentiation-dependent manner (Bello-Morales et al., 2014). Furthermore, in this context and depending on the differentiation stage of the oligodendrocytes, the virus exhibited diverse entry pathways and the small GTPase Rab27a, involved in exocytosis regulation and membrane trafficking events, might play an important role during diverse processes of the viral cycle (Bello-Morales et al., 2012; Bello-Morales et al., 2014).

In this study, we have investigated the effect of VPA as a plausible inhibitor of the infection of HOG cells by HSV-1, a suitable model to study many aspects of the antiviral and cellular toxicity of this drug along the cellular maturation pathway. Herein, our results indicate that VPA treatment of HOG cells significantly reduces the effect of HSV-1 infection, virus entry and productivity without affecting cellular viability.

2. Materials and methods

2.1. Cell lines and virus

The human HOG cell line, established from a surgically removed human oligodendroglioma (Post and Dawson, 1992) was kindly provided by Dr. A. T. Campagnoni (University of California, UCLA, USA). Cells were cultured on Petri dishes in Growth Medium (GM) containing low-glucose DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (50 U/mL) and streptomycin (50 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2. To induce differentiation, cells were cultured in serum-free Differentiation Medium (DM) containing low-glucose DMEM as described (Bello-Morales et al., 2014).

HSV-gL86 is a betta-galactosidase-expressing recombinant virus containing Lac-Z gene from *Escherichia coli* (Montgomery et al., 1996). This virus was a kind gift of Dr. R. Longnecker (North-western University, Chicago, USA). After entry into cells, like R120vGF, this virus expresses betta-galactosidase protein and is able to complete the viral cycle. This virus was used only in cell entry assays.

HSV-R120vGF is an EGFP-expressing recombinant virus containing vhs gene and immediate early genes except ICP4 (Bello-Morales et al., 2014). This virus was propagated in E5 cells, a Vero cell line expressing the ICP4 protein of HSV-1 (DeLuca et al., 1985). After entry into cells, HSV-R120vGF virus expresses EGFP and immediate early proteins, but it is not able to complete the viral cycle due to the lack of ICP4.

HSV-K26GFP virus was a kind gift of Dr. Desai (Johns Hopkins University, Baltimore, USA). It was obtained by fusing GFP to the HSV-1 capsid late protein VP26 (Desai and Person, 1998). HSV-K26GFP wild type HSV-1 (F strain) viruses were propagated and titrated on Vero cells.

2.2. Cell viability assay in presence of VPA

The effect of VPA in HOG cells was analysed by the MTT method (Roche-11465007001). Non confluent monolayers of HOG cells plated in 96-well tissue culture dishes were incubated for 17 or 48 h with different concentrations of VPA in GM medium. Then, cells were incubated with a final concentration of 0.5 mg/mL MTT in a humidified atmosphere for 4 h. The formazan crystals formed were solubilized in 10% SDS in 0.01 M HCl. The resulting colored solution was quantified using a scanning multiwall spectrophotometer (ELISA reader). The wavelength to measure absorbance of the formazan product was 595 nm.