Antiviral Research 137 (2017) 76–81

Contents lists available at ScienceDirect

Antiviral Research

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

The natural compound silvestrol is a potent inhibitor of Ebola virus replication

Nadine Biedenkopf [a, d, 1], Kerstin Lange-Grünweller [b, 1], Falk W. Schulte [b], Aileen Weißer [b], Christin Müller [c, d], Dirk Becker [a, d], Stephan Becker [a, d], Roland K. Hartmann [b], Arnold Grünweller [b, *]

[a] Institut für Virologie, Philipps-Universität Marburg, Hans-Meerwein-Str. 2, 35043, Marburg, Germany
[b] Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, 35037, Marburg, Germany
[c] Institut für Medizinische Virologie, Justus-Liebig-Universität Gießen, Schubertstraße 81, 35392, Gießen, Germany
[d] Deutsches Zentrum für Infektionsforschung (DZIF) at the Partner Site Gießen-Marburg-Langen, Germany

ARTICLE INFO

Article history:
Received 19 August 2016
Received in revised form
10 November 2016
Accepted 11 November 2016
Available online 15 November 2016

Keywords:
Ebola
Silvestrol
eIF4A helicase
Translation inhibition
Primary macrophages
PIM1

ABSTRACT

The DEAD-box RNA helicase eIF4A, which is part of the heterotrimeric translation initiation complex in eukaryotes, is an important novel drug target in cancer research because its helicase activity is required to unwind extended and highly structured 5′-UTRs of several proto-oncogenes. Silvestrol, a natural compound isolated from the plant *Aglaia foveolata*, is a highly efficient, non-toxic and specific inhibitor of eIF4A. Importantly, 5′-capped viral mRNAs often contain structured 5′-UTRs as well, which may suggest a dependence on eIF4A for their translation by the host protein synthesis machinery. In view of the recent Ebola virus (EBOV) outbreak in West Africa, the identification of potent antiviral compounds is urgently required. Since Ebola mRNAs are 5′-capped and harbor RNA secondary structures in their extended 5′-UTRs, we initiated a BSL4 study to analyze silvestrol in EBOV-infected Huh-7 cells and in primary human macrophages for its antiviral activity. We observed that silvestrol inhibits EBOV infection at low nanomolar concentrations, as inferred from large reductions of viral titers. This correlated with an almost complete disappearance of EBOV proteins, comparable in effect to the translational shutdown of expression of the proto-oncoprotein PIM1, a cellular kinase known to be affected by silvestrol. Effective silvestrol concentrations were non-toxic in the tested cell systems. Thus, silvestrol appears to be a promising first-line drug for the treatment of acute EBOV and possibly other viral infections.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The rocaglate silvestrol, a natural compound with a cyclopenta*[b]*benzofuran skeleton, can be isolated from the plant *Aglaia foveolata* (Kim et al., 2007) and is a potent and highly specific inhibitor of the ATP-dependent DEAD-box RNA helicase eIF4A (Bordeleau et al., 2008). This helicase is part of the heterotrimeric translation initiation complex eIF4F that binds to m^7GpppN mRNA cap structures through its eIF4E subunit, thus enabling the recruitment of ribosomes to the 5′-UTR of mRNAs in eukaryotes (Pelletier et al., 2015). It has been proposed that silvestrol increases the affinity of eIF4A for the bound target mRNA, thereby stalling the helicase on its RNA substrate, which leads to a depletion of eIF4A from mRNA-bound eIF4F complexes (Sadlish et al., 2013). Silvestrol exerts potent and non-toxic antitumor activity *in vitro* and *in vivo* (Lucas et al., 2009; Kogure et al., 2013) by inhibiting translation of short-lived oncoproteins, such as c-MYC and PIM1 (Schatz et al., 2011), whose mRNA 5′-UTRs are extended and include regions of stable RNA secondary structures that require unwinding by eIF4A to create a binding platform for the 43S preinitiation complex (Hinnebusch et al., 2016).

The recent outbreak of the Ebola virus (EBOV) in West Africa caused more than 28,000 cases with at least 11,000 fatalities, revealing that effective viral inhibitors with potential broad-spectrum activity are urgently needed (WHO Ebola Response Team et al., 2015). EBOV is a negative-stranded RNA virus whose 19-kb genome comprises seven protein genes. Transcription of the viral genes is accomplished by the viral polymerase complex which synthesizes monocistronic, 5’-capped and 3’-polyadenylated

* Corresponding author.
E-mail address: gruenwel@staff.uni-marburg.de (A. Grünweller).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.antiviral.2016.11.011
0166-3542/© 2016 Elsevier B.V. All rights reserved.

mRNAs (Sanchez et al., 1993; Mühlberger et al., 1999). As for all viruses, EBOV protein synthesis depends on the cellular translation machinery. In this context, antivirals that target host factors are expected to be an advantageous strategy compared to viral targets because escape mutations by the virus are rarer (Müller et al., 2012). However, inhibition of host factors is often a problematic issue in drug development due to pleiotropic unwanted side-effects associated with such strategies (Gerold and Pietschmann, 2013). This concern appears to be mitigated in the case of silvestrol as its inhibition of eIF4A was shown to be highly specific, resulting in efficient and non-toxic antitumor activity in different tumor mouse model systems (Lucas et al., 2009; Kogure et al., 2013). Therefore, eIF4A is discussed as a promising new drug target for cancer treatment (Chu and Pelletier, 2015).

Interestingly, some EBOV mRNAs harbor long 5′-UTRs and all EBOV mRNAs were predicted (Mühlberger et al., 1999) or demonstrated (Weik et al., 2002; Schlereth et al., 2016) to adopt stable RNA secondary structures in their 5′-UTRs. We thus hypothesized that efficient cap-dependent translation of EBOV mRNAs may also require the host helicase activity of eIF4A to unwind such structures during initiation of translation. To test our hypothesis, we analyzed potential inhibitory effects of silvestrol on EBOV-infected Huh-7 cells as well as primary human macrophages, the primary target cells of EBOV in humans.

2. Materials and methods

2.1. Cell culture and preparation of macrophages from PBMCs

Huh-7 (human hepatoma) and VeroE6 (African green monkey, kidney epithelia) cells were cultivated in Dulbecco's modified Eagle medium complemented with penicillin (100 U/ml), streptomycin (100 mg/ml), 5 mM L-glutamine and 10% fetal calf serum at 37 °C and 5% CO_2 in a humidified atmosphere.

Human PBMCs (Peripheral Blood Mononuclear Cells) were isolated from buffy coats by density gradient centrifugation and magnetic CD14 MicroBeads according to the manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes (3×10^6 cells) were cultivated in 6-well tissue culture plates (Falcon Primaria, Becton Dickinson, Paramus, NJ) at 37 °C in 5% CO_2 using RPMI 1640 medium (GE Healthcare, Freiburg, Germany) supplemented with 2 mM L-Glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin and 1% non-essential amino acids. After 2 h, defibrinated human AB serum was added to a final concentration of 2%, and GM-CSF (Biochrom, Berlin, Germany) to a final concentration of 10 ng/ml. Cells were cultivated for 7 days to differentiate them into macrophages. On day 3 post seeding half of the medium was replaced with fresh medium (including all supplements). Alternatively, preparation of monocytes from PBMCs was performed by counterflow centrifugation.

2.2. Ebola virus infection

All work with Zaire Ebola virus (EBOV) strain Mayinga (Acession number AF 086833) was performed at the biosafety level 4 (BSL4) laboratory of the Philipps University Marburg. Briefly, Huh-7 cells (5×10^5 cells) or primary human macrophages (3×10^6 cells) were infected with EBOV at a multiplicity of infection (MOI) of 0.1 for 1 h at 37 °C. Inoculum was removed and cells were incubated in Dulbecco's modified Eagle medium (DMEM) containing 3% fetal calf serum supplemented with silvestrol or Dimethylsulfoxid (DMSO) as indicated.

Aliquots of the supernatant were collected at 1–4 days post infection (p.i.) and subjected to the 50% tissue culture infectious dose $TCID_{50}$ assay for determination of viral titers. Cells were lysed with 1% SDS, followed by Western blotting to analyze for expression of viral proteins and the cellular PIM1 kinase.

2.3. Silvestrol treatment of EBOV-infected cells

Silvestrol (Medchemexpress LLc, Princeton, USA; purity >98%) was added to cell cultures as a single dose to final concentrations of 1–50 nM (dissolved from a 6 mM stock in DMSO, dilutions in DMEM). Silvestrol was added (Huh-7: one day post seeding; primary human macrophages: 7 days post seeding) 2 h before infection or directly post infection. As a control, DMSO was added to cells at the same concentration used to dissolve silvestrol. Infected cells were incubated for 1–4 days at 37 °C in growth medium containing the indicated silvestrol concentrations.

2.4. $TCID_{50}$ analysis

Virus titers in the supernatant of infected cells were determined by the 50% tissue culture infectious dose ($TCID_{50}$) assay in VeroE6 cells as described previously (Krähling et al., 2010).

2.5. WST-1 assay of human Huh-7 cells or primary macrophages

Huh-7 cells (7×10^3 cells in 200 μL Iscove's Modified Dulbecco's Medium [IMDM], 10% fetal calf serum [FCS]) were seeded in 96-well microplates and incubated for 24 h (37 °C, 5% CO_2). The medium was replaced with fresh medium, and silvestrol or DMSO was added as indicated for 48 h. The medium was aspirated and 110 μL of 10% WST-1 reagent (Roche, Mannheim, Germany) in PBS were added. Absorbance was measured using a Tecan Safire II (measurement wavelength: 450 nm; reference wavelength: 600 nm).

Monocytes (8×10^4 cells in 100 μL RPMI 1640) were seeded in 96-well microplates and incubated for 2 h (37 °C, 5% CO_2). Then 100 μL RPMI 1640 supplemented with defibrinated human AB serum (final concentration 2%) and GM-CSF (final concentration 10 ng/ml) (Biochrom, Berlin, Germany) were added. After 72 h, half of the medium was removed and replaced with RPMI 1640 containing 4% human serum. After 96 h, the monocytes had differentiated into macrophages. The medium was then replaced with RPMI 1640 containing 2% human serum, and silvestrol or DMSO was added as indicated followed by incubation for another 72 h. The WST-1 assay was then performed after 24, 48, 72 and 96 h as described above for Huh-7 cells.

2.6. Western blot analysis

Cells were lysed in lysis buffer (125 mM Tris/HCl pH 6.8, 4% SDS, 1.4 M 2-mercaptoethanol, 0.05% bromophenol blue) and heated at 95 °C for 5 min. Samples were loaded onto 15% SDS-polyacrylamide gels followed by electrophoresis for 1 h at 180 V. Proteins were transferred onto an Immobilon-P PVDF membrane (Merck Millipore, Darmstadt, Germany) for 30 min at 10 V followed by blocking of the membrane with 5% milk powder dissolved in TBST (10 mM Tris/HCl, 150 mM NaCl, 0,1% Tween 20, pH 7.6). Primary and secondary antibodies were diluted 1:500 in TBST (Pim1, sc-13513, Santa Cruz Biotechnology), 1:5000 (β-Actin, sc-47778, Santa Cruz Biotechnology) and 1:5000 (goat anti-mouse IgG-HRP, secondary antibody). Blots were incubated with Amersham ECL™ or ECL-plus™ Western Blotting Detection Reagents according to the manufacturer's protocol. For detection of chemiluminescence, Kodak® BioMaxTM light films, Kodak GBX Developer and Replenisher and GBX Fixer and Replenisher were used.

Samples from infected cells were lysed in 1% SDS containing sample buffer and subjected to 12% SDS PAGE and semi-dry transfer onto nitrocellulose membranes. Staining of viral proteins from

Download English Version:

https://daneshyari.com/en/article/5551905

Download Persian Version:

https://daneshyari.com/article/5551905

Daneshyari.com