Polish Journal of Veterinary Sciences Vol. 16, No. 4 (2013), 749–751

DOI 10.2478/pjvs-2013-0106

Short communication

Influence of importin α/β and exportin 1 on equine herpesvirus type 1 (EHV-1) replication in primary murine neurons

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Abstract

Viruses replicating in the nucleus need to cross the nuclear membrane barrier during infection, therefore disruption of specific nuclear transport pathways is crucial for their replication cycle. In the present study we have investigated the influence of nucleo-cytoplasmic transport inhibitors – ivermectin and leptomycin B, on EHV-1 replication in primary murine neurons. Obtained results suggest that the examined proteins – exportin 1 and importin α/β may participate, but are not required, during EHV-1 infection. Based on these results, it can be assumed that EHV-1 is able to use other receptors for nucleo-cytoplasmic transport.

Key words: EHV-1, ivermectin, leptomycin B, exportin 1, importin α/β

Introduction

Equine herpesvirus type 1 (EHV-1) is a member of the alphaherpesviruses that replicate and encapsidate their genome within the nucleus of infected cell. To reach the sites of viral replication, many viruses have evolved a variety of interactions with host cells to exploit the cytoskeleton (involved in intracellular transport), as well as nucleo-cytoplamic trafficking machinery. Receptor-mediated nuclear import and export is utilized by adenoviruses, parvoviruses, flaviviruses, retroviruses and herpesviruses and disruption of specific nuclear transport pathways is crucial for the efficacious replication cycle (Greber and Fornerod, 2004, Mastrangelo et al. 2012, Wagstaff et al. 2012). In the present study, we have used two inhibitors – ivermectin as an importin α/β inhibitor and leptomycin B as an exportin 1 inhibitor to determine their effect on EHV-1 replication in primary murine neurons.

Materials and Methods

Cell culture and viruses

Primary culture of murine neurons was established as described before (Cymerys et al. 2010). Neuronal cells were suspended in B-27 Neuron Plat-

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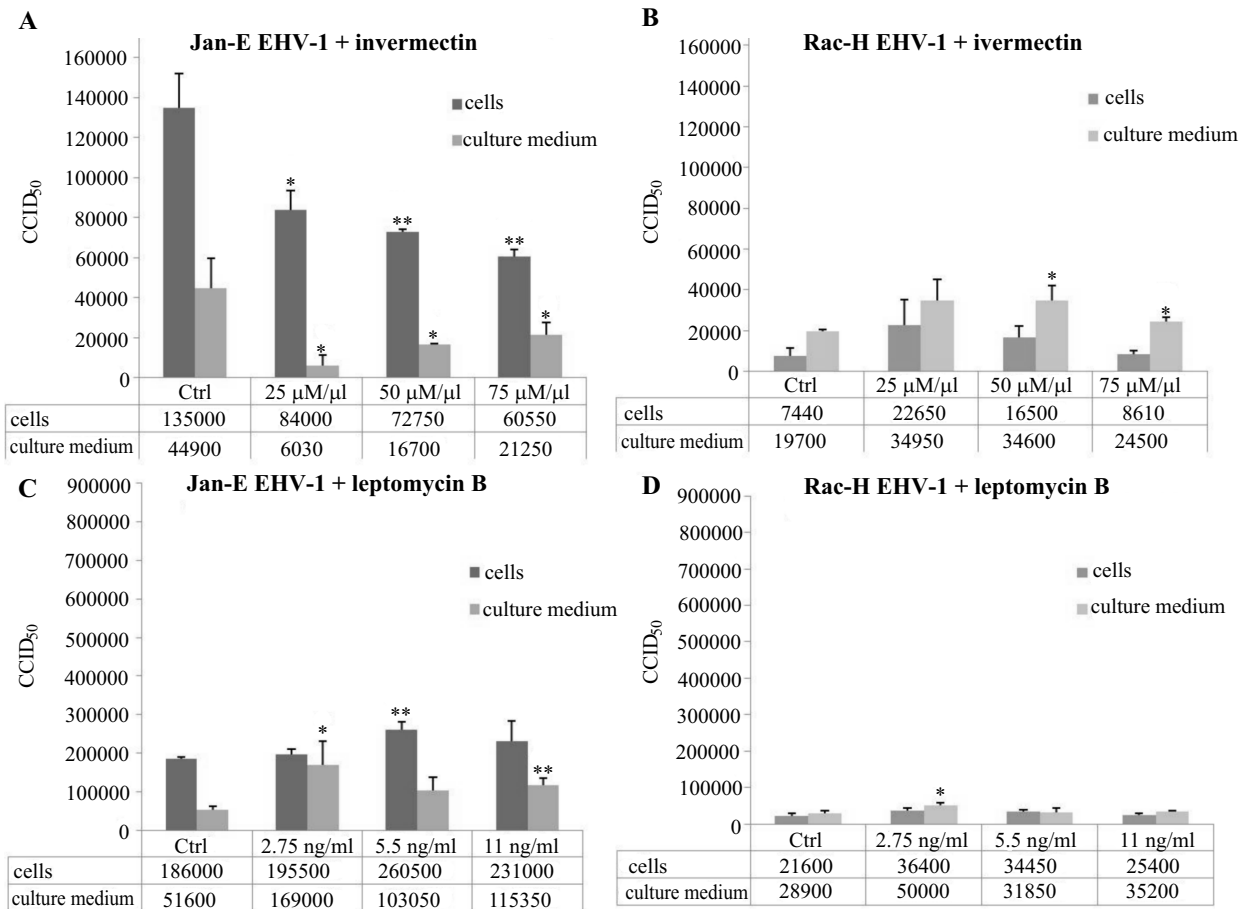

Fig. 1. The influence of ivermectin and leptomycin B on EHV-1 replication in primary murine neurons (24 h.p.i). Comparison of the viral DNA ($CCID_{50}$) in cells and culture medium.

ing Medium consisting of neurobasal medium, B27 supplement, glutamine (200 mM), glutamate (10 mM), antibiotics (penicillin and streptomycin) with 10% supplement of fetal and equine serum (Gibco) and plated onto 12-well plates (10^6 cells/ml). Two strains of EHV-1 from the virus collection of the Virology Laboratory at Warsaw University of Life Sciences – SGGW were used: (i) strain Jan-E isolated from aborted fetus (12^{th} passage in ED cells) and (ii) strain Rac-H, which has been passaged through a series of cell cultures and is defined as pantropic, non-pathogenic. Primary murine neuronal cells were infected with approx. 10^5 $CCID_{50}$/ml (MOI=0.3) of Jan-E or Rac-H strain and incubated for 24 hours at 37°C with 5% CO_2.

Inhibitor treatment and real-time PCR

Ivermectin and leptomycin B (Sigma Chemicals), were used to disrupt nuclear import and export, respectively. Neuronal cells were pretreated for 4 h with 25, 50 or 75 µM/ml ivermectin before EHV-1 infection and inhibitor remained in the culture medium. Leptomycin B was added to the culture medium after infection at a final concentration of 2.75, 5.5 or 11 ng/ml. At 24 h p.i., viral DNA of appropriate material (cells or culture medium), was isolated using High Pure Viral Nucleic Acid Kit (Roche Diagnostics), according to manufacturer's instructions, and analyzed using real-time PCR technique according to the in-house quantitative method (Dzieciątkowski et al. 2009).

Results and Discussion

Recently, many studies have been devoted to nuclear transport inhibitors as an invaluable tool for studying nuclear import and export, as well as the basis for future development of antiviral agents. Therefore, in the present study we have investigated the influence of ivermectin and leptomycin B on the EHV-1 replication in primary murine neurons. Treatment of murine neurons with different concentrations of ivermectin had no effect on the replication of

Rac-H EHV-1 (Fig. 1B), however it diminished Jan-E EHV-1 replication (Fig. 1A). These data demonstrated that used EHV-1 strains were able to employ various receptors for nuclear import, while importin α/β participated, but was not essential in this process. Moreover, we have not observed significant changes in EHV-1 replication in murine neurons in the presence of a wide range of leptomycin B concentrations (Fig. 1 C-D), thus it can be assumed that EHV-1 was not dependent on the exportin 1-mediated nuclear export. Many studies have previously confirmed that ivermectin is able to inhibit replication of HIV-1, dengue virus and West Nile virus (Mastrangelo et al. 2012, Wagstaff et al. 2012). It has been demonstrated that ivermectin is affecting NS3 helicase activity, required during viral RNA replication. However, in our studies suppression of replication cycle was observed only for Jan-E EHV-1 strain. Thus, establishing a potent antiviral activity towards EHV-1 requires further research. In contrast to our findings, it has been reported that in the presence of leptomycin B viral infection was blocked and Miller and Pintel (2002) demonstrated that leptomycin B treatment of MVM-infected cells resulted in retention of assembled capsids in the nucleus of treated cells. Based on these results it can be assumed that exportin 1 and importin α/β may participate, but are not actually required during EHV-1 infection and the virus is able to use other receptors required for nucleo-cytoplasmic transport to accomplish replication cycle.

Acknowledgements

This work was supported by the grant No. NN308 028 739 from Polish Ministry of Science and Higher Education.

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