Antiviral Research 123 (2015) 105–113

Contents lists available at ScienceDirect

Antiviral Research

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

Valacyclovir combined with artesunate or rapamycin improves the outcome of herpes simplex virus encephalitis in mice compared to antiviral therapy alone

Coraline Canivet, Rafik Menasria, Chantal Rhéaume, Jocelyne Piret, Guy Boivin *

Research Center of the CHU of Quebec and Laval University, Quebec City, QC, Canada

ARTICLE INFO

Article history:
Received 9 July 2015
Revised 10 September 2015
Accepted 11 September 2015
Available online 14 September 2015

Keywords:
Herpes simplex virus encephalitis
Inflammation
Antiviral agents
Immunomodulatory drugs
Artesunate
Rapamycin

ABSTRACT

Despite antiviral therapy, the mortality rate of herpes simplex virus encephalitis (HSE) remains high and many surviving patients harbor neurological sequelae. Although viral replication is responsible for substantial neurological damages, an exaggerated inflammatory response could also contribute to this process. Artesunate (ART) and rapamycin (RAPA) have shown some benefits in the treatment of herpes simplex virus infections. Herein, we evaluated the benefit of combining ART or RAPA with valacyclovir (VACV) in a murine model of HSE. Infected mice were treated with VACV (1 mg/mL in drinking water) from day 3 post-infection (p.i.) combined or not with daily intraperitoneal administration of ART (30 mg/kg) or RAPA (20 mg/kg) from days 4 to 13 p.i. Viral load, infectious titers, cytokine and chemokine levels were measured in brain homogenates on days 5, 7 and 9. The survival rates of mice treated with VACV and ART or RAPA were higher than with VACV alone (71.9% versus 43.2% for ART and 66.7% versus 43.2% for RAPA; both $P \leqslant 0.05$) but no significant difference was seen in the brain viral loads. Levels of IL-1β, IL-2 (both $P \leqslant 0.05$), IL-6, IFN-γ (both $P \leqslant 0.01$), CCL2 ($P \leqslant 0.01$), CCL3 and CCL4 (both $P \leqslant 0.05$) were reduced in mice treated with VACV combined with ART versus VACV alone. Levels of IL-6, IL-1β and IFN-γ slightly increased on day 7 in mice treated with VACV combined with RAPA compared to VACV alone and then decreased on day 9. Our results suggest that immunomodulatory compounds such as ART or RAPA could benefit antiviral therapy in HSE.

1. Introduction

Herpes simplex virus encephalitis (HSE) is the most common cause of lethal viral encephalitis in Western countries (Tyler, 2004). The annual incidence of HSE is estimated to be 5 cases per million individuals and herpes simplex virus (HSV) 1 is responsible for 90% of cases (Rozenberg et al., 2011). HSE consists in a diffuse inflammatory process in brain parenchyma associated with signs of cerebral dysfunctions. In the absence of antiviral therapy, the mortality rate associated with HSE approaches 70%. Despite early administration of acyclovir (ACV), the mortality rate of HSE is still close to 30% with nearly 60% of survivors developing neurological sequelae. The pathogenesis of HSE is not well understood. After intranasal infection of mice with HSV-1, a prolonged microglial activation was observed and, macrophages and neutrophils rapidly infiltrated the brain followed by lymphocytes, largely composed of $CD8^+$ T cells (Marques et al., 2008). It is thus suggested that direct virus-related and indirect immune-mediated mechanisms contribute to the damages occurring in the central nervous system (CNS) during HSE. In this regards, corticosteroids have been reported to improve the outcome of HSE in a murine model by reducing the activation of several inflammatory pathways (Sergerie et al., 2007). A recent review also suggested that adjunctive corticosteroid therapy may have beneficial effects in the treatment of patients suffering from HSE (Ramos-Estebanez et al., 2014).

Innate immune response constitutes the first line of host defense that limits viral spread. Toll-like receptor (TLR) 2, TLR3 and TLR9 have been reported to play a major role in the pathogenesis of HSE in murine models (Kurt-Jones et al., 2004; Reinert et al., 2012; Sorensen et al., 2008; Wang et al., 2012). TLR2 is located on the cell surface and has been shown to sense viral components such as glycoproteins H, L or B (Leoni et al., 2012). TLR3 and TLR9 are found in the endosomes and respectively participate in the recognition of double-stranded RNA intermediates produced during viral replication and unmethylated CpG DNA

* Corresponding author at: Research Center in Infectious Diseases, CHU de Québec – CHUL, 2705 Blvd Laurier, RC-709, Quebec City, QC G1V 4G2, Canada.
E-mail address: guy.boivin@crchudequebec.ulaval.ca (G. Boivin).

http://dx.doi.org/10.1016/j.antiviral.2015.09.007
0166-3542/

(Krug et al., 2004; Weber et al., 2006). After activation, these TLRs can trigger multiple cellular responses including the production of pro-inflammatory cytokines and chemokines that play an important role in the activation of the adaptive immunity.

Artesunate (ART) is commonly used in the treatment of severe malaria. ART has also been reported to be effective against multidrug-resistant infections caused by HSV-2 in hematopoietic stem cell transplant recipients (Sellar et al., 2012). Previous studies have demonstrated that ART exerts an immunomodulatory activity by modulating TLR2 and TLR9 pathways in murine models of bacterial infections (Li et al., 2008, 2010). On the other hand, rapamycin (RAPA), an inhibitor of the mammalian target of rapamycin (mTOR), is a widely used immunosuppressive drug. RAPA acts by inhibiting several signal transduction pathways in cytokine-stimulated T cells and blocks the cell-cycle progression from G1 to S phase. This leads to an inhibition of the clonal expansion and cytotoxic activity of T lymphocytes (Janes and Fruman, 2009; Powell et al., 1999). In mouse models of herpetic stromal keratitis, RAPA was shown to reduce lesions severity by decreasing the inflammatory response (Deshpande et al., 2001; Zapata et al., 2012). The use of ART or RAPA to respectively inhibit TLR2 and TLR9 activation or T cell functions at a critical time after infection could thus reduce the overzealous inflammatory response that develops during HSE.

In this study, we evaluated the added benefit of combining ART or RAPA with valacyclovir (VACV) in a murine model of HSE by analyzing the effects of individual or combined treatments on the survival rate as well as on the viral DNA load, infectious viral titers and pro-inflammatory cytokine and chemokine levels in brain homogenates at different times post-infection.

2. Materials and methods

2.1. Drugs

ART and ACV were purchased from Sigma–Aldrich (Oakville, ON, Canada). Rapamycin and VACV were respectively obtained from Biorbyt (San Francisco, CA) and GlaxoSmithKline (Mississauga, ON, Canada).

2.2. Drug susceptibility assays

The activity of ART, RAPA and ACV against the HSV-1 strain H25 was evaluated in NIH/3T3 cells (mouse embryonic fibroblasts; CRL-1658™ ATCC®, Manassas, VA) by the plaque reduction assay (Swierkosz et al., 2004). Drugs were added to cells 30 min before and left during and after infection (pre-treatment) or added 90 min after cell infection (post-infection).

2.3. Animals and experimental procedures

Four- to five-week-old female BALB/c mice were purchased from Charles River Canada (St-Constant, QC, Canada). All experimental procedures were approved by the Animal Care Ethics Committee of Laval University. In a first set of experiments, the effect of ART and RAPA alone was evaluated in a low viral inoculum model of HSE. Mice were infected intranasally with 1.5×10^3 plaque forming units (PFU) of the neurovirulent clinical HSV-1 strain H25 described elsewhere (Sergerie et al., 2007). Viral titers were determined in Vero cells after virus stock production and at the end of each experiment (back titration). Mice were treated by daily intraperitoneal (ip) administration of the vehicle (0.9% saline), 15 mg/kg of ART or 10 mg/kg of RAPA from days 4 to 13 post-infection (p.i.). For survival rate experiments, mice were monitored during 21 days for appearance of HSE-related signs (i.e., weight loss, isolation with no social interaction, limited movements, swollen eyes, neurological disorders, convulsion and mortality). Animals were sacrificed when a weight loss $\geqslant$20% or a combination of two other obvious sickness signs were recorded. To evaluate the effect of ART and RAPA on viral load, subsets of mice were sacrificed on days 5 and 7 p.i. and brain homogenates were prepared as previously described (Boivin et al., 2012b).

In a second set of experiments, the effect of VACV alone or combined with ART or RAPA was evaluated in a high viral inoculum model of HSE. Mice were infected intranasally with 1.5×10^4 PFU of HSV-1 strain H25. Mice were treated with VACV (1 mg/mL in drinking water *ad libitum* from day 3 and on) as this dosage was previously demonstrated to be more effective than twice daily administration of 50 mg/kg by oral gavage (Field and Thackray, 1995). VACV treatment was combined or not with daily ip administration of the vehicle, ART (30 mg/kg) or RAPA (20 mg/kg) from days 4 to 13 p.i. For survival rate experiments, mice were monitored during 21 days for appearance of HSE-related signs as described above. Subsets of mice were sacrificed prior to infection and on days 5, 7 and 9 p.i. and brain homogenates were prepared as reported (Boivin et al., 2012b) to determine viral DNA load, infectious titers, cytokine and chemokine levels.

2.4. Viral DNA load and infectious titers measurements

Total DNA was extracted from 10 mg of brain homogenates using the MagNA Pure LC DNA Isolation Kit II (Roche Molecular System, Laval, QC, Canada) and eluted in 200 μL of elution buffer according to manufacturer's instructions. Real-time PCR was performed using 5 μL of extracted total DNA and the LightCycler® 480 Probes Master mix in a LightCycler® 480 system (both from Roche Molecular System). External standards were run in parallel as described elsewhere (Boivin et al., 2006). Primers and probes targeted a conserved region of the DNA polymerase of HSV-1. Infectious titers were determined in brain homogenates by a standard plaque assay on Vero cells (Boivin et al., 2012b). The limit of detection of the assay was 5 PFU per well.

2.5. Cytokine and chemokine levels measurements

Brain homogenates were centrifuged at 10,000×*g* for 10 min at 4 °C. IFN-β protein levels were measured in supernatants by ELISA (Life Technologies, Burlington, ON, Canada). Levels of IFN-γ, IL-1β, IL-2, IL-6, keratinocyte chemoattractant (KC), chemokine ligand (CCL)-2, CCL3, CCL4 and CCL5 were determined in supernatants by magnetic bead-based immunoassays using the Bio-Plex mouse cytokine group I plex assay (Bio-Rad Laboratories, Mississauga, ON, Canada) according to the manufacturer's instructions. Data were analyzed using a Bio-Plex system equipped with the BioPlex Manager Software v6.0.

2.6. Statistical analyses

All statistical analyses were performed using GraphPad Prism software program v5 (GraphPad Software, San Diego, CA). Differences in survival rates were analyzed using a Log-Rank (Mantel–Cox) test. Differences in mean life expectancies were analyzed using a non-parametric analysis of variance (ANOVA) with Dunn's post-test for multiple group comparison. Differences in weight changes were analyzed using a two-way ANOVA with Bonferroni post-test. Differences in viral DNA load, infectious titers, cytokine and chemokine levels were analyzed by a one-way ANOVA with Tukey's multiple comparison post-test after logarithmic transformation of the data. A P value $\leqslant$0.05 was considered as statistically significant.