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Acyclovir-resistant herpes simplex virus type 1 in intra-ocular fluid samples of herpetic uveitis patients

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ABSTRACT

Background: Acyclovir (ACV) is the antiviral drug of choice to treat patients with herpes simplex virus type 1 (HSV-1) uveitis. The prevalence of intra-ocular ACV-resistant (ACV^R) HSV-1 in herpetic uveitis is unknown and may have clinical consequences. In addition to its predictive value on ACV susceptibility, the polymorphic HSV-1 thymidine kinase (TK) gene facilitates differentiation between HSV-1 strains.
Objectives: The objective of this study was to determine the genetic composition and ACV susceptibility of the causative virus in intra-ocular fluid samples (IOF) of HSV-1 uveitis patients.
Study design: The intra-ocular HSV-1 pool from 11 HSV-1 uveitis patients was determined by sequencing IOF-derived viral TK genes. The ACV susceptibility profile of the cloned intra-ocular TK variants was defined by mass spectrometry. In addition, the ganciclovir (GCV) susceptibility of the ACV^R HSV-1 TK variants was defined.
Results: Intra-ocular fluid samples of HSV-1 uveitis patients contain HSV-1 quasispecies, principally consisting of one major and multiple genetically related minor patient-specific TK variants. Four of 10 patients analyzed had an intra-ocular ACV^R HSV-1 of which 3 were cross-resistant to GCV. The ACV^R profile of intra-ocular HSV-1 did not correlate with symptomatic ACV treatment.
Conclusions: Affected eyes of HSV-1 uveitis patients are commonly infected with a patient-specific HSV-1 quasispecies, including one major and multiple genetically related minor variants. A relatively high prevalence of intra-ocular ACV^R HSV-1, mainly ACV/GCV cross-resistant viruses, was detected in HSV-1 uveitis patients.

1. Background

Herpes simplex virus type 1 (HSV-1) is an endemic human alphaherpesvirus that causes a variety of diseases, including the sight-threatening ocular diseases keratitis and uveitis.[1,2] HSV-1 establishes a lifelong latent infection in sensory neurons that innervate the site of primary infection and reactivates intermittently to cause recrudescent disease.[1]

HSV-1 uveitis is due to an initial cytopathic effect of the virus on uveal-resident cells followed by a local inflammatory response to the inciting virus. Treatment is therefore aimed at inhibiting viral replication with antiviral drugs and dampening the intra-ocular immune response with anti-inflammatory agents.[2] The antiviral drug acyclovir (ACV), highly selective for human alphaherpesviruses and low in cytotoxicity, is the gold standard to treat HSV-1 uveitis. Acyclovir is converted to ACV-monophosphate (ACVmp) by virus-encoded thymidine kinase (TK) protein, which is subsequently converted by cellular enzymes to the active compound ACV-triphosphate that inhibits viral replication.[3,4] ACV can be applied topically and systemically and is given as prophylaxis to prevent recurrent ocular HSV-1 diseases.[5]

The prevalence of ACV resistant (ACV^R) HSV-1 is generally low for immunocompetent individuals (<1%), but higher in the immunocompromised (4–14%).[3,6–8] This difference illustrates the pivotal role of the host's immune system to control HSV-1 infections.[4] Recently, we reported on the relatively high incidence of ACV^R HSV-1 in otherwise healthy HSV-1 keratitis patients.[7]

Abbreviations: HSV-1, herpes simplex virus type 1; IOF, intra-ocular fluid; TK, thymidine kinase; ACV, acyclovir; GCV, ganciclovir; TG, trigeminal ganglion.

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Notably, patients with corneal ACV^R HSV-1 were refractory to ACV therapy and had poor visual outcome.[7,9] Resistance to ACV is commonly due to specific mutations in the viral TK and incidentally in the viral DNA polymerase gene. ACV^R HSV-1 viruses may have a deficient TK protein or altered substrate activity.[3,4,10,11] Moreover, the natural hypervariability of the HSV-1 TK gene facilitates differentiation of HSV-1 strains.[12,13]

2. Objectives

The prevalence of intra-ocular ACV^R viruses in HSV-1 uveitis is unknown and may have clinical consequences. The objective of this study was to determine the genetic composition and ACV susceptibility of intra-ocular HSV-1 in herpetic uveitis patients. Additionally, we determined the cross-resistance of intra-ocular ACV^R HSV-1 to ganciclovir (GCV).

3. Study design

3.1. Clinical specimens

The samples analyzed were surplus intra-ocular fluid (IOF) samples, either aqueous humor or vitreous fluid, obtained from 11 immunocompetent uveitis patients during diagnostic paracentesis or therapeutic vitrectomy (patients UV2, UV5 and UV7), respectively (Table 1). HSV-1 was identified as the causative virus by real-time PCR analysis on the respective IOF samples.[14] The clinical variables scored were age at sampling date, gender, ACV therapy regimen and clinical picture of ocular disease at presentation and in the preceding years. No detailed clinical information of patient UV3 was available. The median patient age was 49 years (range 30–69 years) and 7 patients were female. Eight patients (73%) received systemic ACV ($n=4$) or valacyclovir (valACV; $n=4$) treatment, of variable duration (3–52 days, average 15 days), directly before IOF sample collection (Table 1). Two patients started valACV therapy after sample collection (UV3 and UV5). Two patients received complementary topical ACV ointment (UV4 and UV11). All patients received topical corticosteroids at time of sampling. After IOF sample collection, systemic (val)ACV treatment was started, continued and/or tapered until the clinical picture improved. None of the patients received topical or systemic ACV treatment in the 2 years preceding sampling and no patients were treated before or during sampling with (val)ACV analogues like ganciclovir (GCV). During follow-up all patients, except for patient UV7, restored visual acuity to baseline levels (data not shown). Patient UV7 lost vision due to ocular hypotonia after therapeutic vitrectomy (Table 1). Study procedures were performed in compliance with Dutch laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

3.2. HSV-1 thymidine kinase sequence analysis

The entire HSV-1 TK gene was amplified by PCR using DNA isolated from IOF samples as described.[7] From 5 patients (UV6–UV10), the intra-ocular HSV-1 TK amplicons were directly used for pool sequencing.[7] From the remaining 6 HSV-1 uveitis patients, the intra-ocular TK gene pool was determined by colony sequencing as described.[12,13] In short, TK amplicons were ligated into the TOPO-TA cloning vector (Invitrogen), transformed into bacteria and a variable number of colonies were sequenced. All sequences were aligned to a reference HSV-1 TK sequence (strain H129; GenBank: GU734772). A maximum-likelihood phylogenetic tree of IOF-derived TK nucleotide sequences was estimated under the general time-reversible model using PhyML 3.0 software. The IOF-derived HSV-1 TK sequences obtained were deposited in GenBank under the accession numbers HQ707581–HQ707642 and JX392955–JX392980 (Table 2 and Supplementary Table 1).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.03.014.

3.3. HSV-1 thymidine kinase functional assay

The functional effect of a selected set of HSV-1 TK variants was assayed by a described mass spectrometry assay.[13] In brief, HSV-1 TK sequences were cloned into the pcDNA3 expression vector (Invitrogen) and expressed as recombinant TK proteins in Cos-7 cells. After 48 h of incubation at 37 °C, TK protein expression was determined by flow cytometry using a HSV-1 TK-specific antibody. Cell lysates were incubated with 100 μM ACV (Sigma–Aldrich) for 0, 30, 60, and 120 min at 37 °C. Acyclovir and ACVmp levels were measured in quintuple by MALDI mass spectrometry.[13,15] ACV/ACVmp-ratios were corrected for the amount of TK protein as measured by flow cytometry (data not shown).[13] The ACV-converting activity was normalized to TK activity derived from the ACV sensitive (ACV^S) HSV-1 reference strain KOS,[13] which was set at 100% after 120 min of incubation. Cut-off value for ACV-resistant TK variants in the HSV-1 mass spectrometry assay was >10% of the TK activity of the ACV-sensitive HSV-1 strain KOS.

3.4. In vitro ganciclovir sensitivity testing

Ganciclovir susceptibility assay used was adapted from a recent study by Shiota et al.[16] In short, a human cornea-derived ACV/GCV cross-resistant HSV-1 strain[7] (GenBank: EU541365) was plaque-purified in vitro under 50 μM ACV and 50 μM GCV (Roche) selection.[17] Cloned IOF-derived HSV-1 TK variants were transfected into Cos-7 cells. After 24 h of incubation at 37 °C, transfected cells were infected with the ACV/GCV cross-resistant HSV-1 clone at a multiplicity of infection of 0.01. Cells were incubated with 0, 0.1, 0.5, 1, 5, 10, and 50 μM of GCV, or solely 50 μM ACV, for 18–24 h at 37 °C. Cell lysate's HSV-1 DNA load was determined by real-time PCR using primers and probes as described.[7,18] An ACV^S HSV-1 (strain KOS) served as positive control for the efficacy of ACV and GCV to inhibit HSV-1 replication. Untransfected Cos-7 cells infected with the ACV/GCV cross-resistant HSV-1 strain served as negative control. All HSV-1 TK variants were assayed in triplicate.

Median inhibitory concentration (IC_{50}) was defined as the concentration of the antiviral drug that reduced viral DNA copies by 50%. HSV-1 TK variant transfection efficiency was checked by TK protein staining and flow cytometry[13] and was always >75% (data not shown). Relative viral replication was normalized per TK variant to the viral replication in the absence of antiviral drugs, set at 100%. HSV-1 TK variants were considered ACV^R or GCV^R if viral replication levels were >1% in the presence of 50 μM ACV and IC_{50} values $\geq$1 μM GCV,[7,18] respectively.

4. Results

4.1. Genetic characteristics of intra-ocular HSV-1 in herpetic uveitis patients

To gain insight into the incidence and clinical significance of intra-ocular ACV^R HSV-1 in HSV-1 uveitis, we determined the genetic composition and ACV susceptibility of IOF-derived HSV-1. Intra-ocular HSV-1 was genotyped by sequencing viral TK genes in IOF samples from 11 HSV-1 uveitis patients. From 6 IOF samples (UV1–UV5 and UV11), the HSV-1 TK gene was PCR amplified, cloned and on average 54 colonies per IOF sample were sequenced (range 45–74 colonies/sample). From the remaining 5 patients (UV6–UV10), the HSV-1 TK amplicon itself was directly used for pool sequencing. In total, 88 of 326 (27%) TK nucleotide