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Surveillance of herpes simplex virus resistance to antivirals: A 4-year survey

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ABSTRACT

Herpes simplex virus (HSV) resistance to antivirals constitutes a therapeutic challenge, especially among immunocompromised patients. This observational survey on HSV resistance to antivirals was conducted retrospectively over a 4-year period (2008–2012). A total of 211 HSV-positive clinical samples (94 HSV-1 and 117 HSV-2) recovered from 139 patients (11 immunocompetent patients, 85 immunocompromised patients, and 43 patients with unknown immune status) with suspected HSV drug-resistance were analyzed for acyclovir and foscarnet susceptibility. Antiviral resistance testing consisted in a two-step procedure including a first-step genotypic assay, based on UL23 (thymidine kinase, TK) and UL30 (Pol) gene sequencing, and a second-step phenotypic assay (i.e., plaque reduction assay) performed when unpreviously described mutations were detected. As a whole, susceptibility and resistance to antivirals were evidenced for 58 (30.7%) and 86 (45.5%) HSV, respectively, whereas antiviral profile remained undetermined for 45 (23.8%) HSV. The prevalence of drug resistance was significantly higher among HSV-2 isolates than among HSV-1 isolates (53.8% *vs.* 34.9%; $p = 0.012$). The majority (i.e., 79.7%) of cases of ACV resistance conferred by TK mutations resulted from UL23 gene frameshift reading. Apart from the changes surely related to natural polymorphism or drug-resistance, 91 unpreviously reported mutations were identified in TK and Pol, including 51 potential natural polymorphisms, 22 mutations likely conferring resistance to antivirals, and 18 mutations of unclear significance.

1. Introduction

The significant morbidity and mortality associated with herpes simplex virus (HSV) infections, requires antiviral treatments to prevent or to cure HSV-associated diseases. UL30 DNA polymerase (Pol) constitutes the viral target of the currently licensed drugs acyclovir (ACV) and penciclovir (PCV), with their respective prodrugs valacyclovir (VACV) and famciclovir (FCV), foscarnet (FOS), and cidofovir (CDV). Viral-encoded UL23 thymidine kinase (TK) is required for ACV and PCV phosphorylation (Piret and Boivin, 2011). Antiviral treatments may result in the emergence of HSV resistance. The prevalence of HSV resistance to antivirals among immunocompetent individuals is low (<1%) (Bacon et al., 2002; Collins and Ellis, 1993). Among immunocompromised patients, this prevalence reaches 3.5% to 10%, with profound immunosuppression and long-term drug exposure as associated risk factors (Danve-Szatanek et al., 2004; Piret and Boivin, 2011). The molecular mechanisms of HSV resistance rely on the presence of mutations within UL23 and UL30 genes. TK alterations, accounting for 95% of HSV resistance to ACV, consist in single-base insertions/deletions, leading to the shift of the translational reading frame of UL23 gene, or missense point mutations. HSV resistance to FOS and/or ACV may be related to mutations within conserved regions of Pol (Piret and Boivin, 2011). Conversely to time-consuming and cumbersome phenotypic antiviral resistance assays, genotypic assays, based on UL23 and UL30 gene sequencing, allow the determination of HSV resistance to antivirals in a clinically relevant time frame (Burrel et al., 2010). However, the involvement of numerous reported mutations in drug-resistance remains to be clearly established. This work reports the results from an observational and retrospective 4-year survey of HSV resistance to antivirals performed in a single center using a genotypic/phenotypic two-step procedure.

2. Materials and methods

2.1. Patients and clinical samples

Between June 2008 and May 2012, clinical samples collected from patients with HSV infection and experiencing incomplete

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virological response despite a well-conducted antiviral treatment (i.e., chronic mucocutaneous lesions and/or persistent viral load) were tested for HSV resistance to antivirals. Each sample was accompanied by a standardized form including: (i) identification of the patient and the hospital department, (ii) age and sex, (iii) immune status, (iv) antiviral treatment, (v) date and site of clinical sample. Samples were tested by real-time PCR assay (Qiagen, Courtaboeuf, France) in order to measure HSV load and to identify HSV type 1 or 2. Remaining sample was frozen at −80 °C for further investigations.

2.2. Antiviral resistance testing procedure

The analysis of HSV resistance to antivirals was performed using the two-step approach implemented in the laboratory. First, a genotypic assay, based on UL23 and UL30 gene sequencing from all HSV-positive samples, allowed the identification of mutations associated with antiviral resistance or natural polymorphism. When the results of the genotypic assay were not conclusive, due to unpreviously described or undefined mutations, a phenotypic assay was performed.

2.3. Genotypic resistance assay

Genotypic resistance assay was performed as previously described (Burrel et al., 2010). Briefly, full-length UL23 and UL30 genes were amplified using HSV type-specific PCR systems with the proofreading enzyme Expand High Fidelity (Roche Diagnostics, Meylan, France). Amplified products were sequenced using overlapping primer pairs with the Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Courtaboeuf, France) and analyzed with the automated sequencer ABI PRISM™ 3730 Genetic Analyser (Applied Biosystems). In order to rule out any PCR artefacts, all sequences were performed twice on both DNA strands. All nucleotide sequences were compared with that of reference strains 17 (HSV-1) and HG52 (HSV-2) (GenBank accession numbers X14112 and Z86099, respectively) using Seqscape v2.5 software (McGeoch et al., 1985; McGeoch et al., 1987).

2.4. Phenotypic resistance assay

HSV isolates were obtained from clinical samples by propagation in Vero cells or human fibroblasts (MRC5). A plaque reduction assay was performed in Vero cell culture for the measurement of the antiviral 50% effective concentration (EC_{50}) towards ACV (Merck, Lyon, France) and FOS (AstraZeneca, Rueil-Malmaison, France). HSV isolates were considered to be resistant at EC_{50} values $\geqslant 7$ μM and 330 μM for ACV and FOS, respectively, as previously reported (Burrel et al., 2010).

2.5. Statistical analyses

Statistical analyses were performed using MedCalc® software. Frequencies and means were compared by use of Fisher's exact test and Student's *t*-test, respectively. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of the study population

During the 4-year period, 211 HSV-positive clinical samples (94 HSV-1 and 117 HSV-2) recovered from 139 patients (median, 1 sample per patient; range, 1–7) were collected for antiviral resistance testing (Table 1). The study population included 73 (52.5%) males and 58 (41.7%) females. The median age was 47 years (range, 3–86) Gender and age were not specified for 8 (5.8%) and 4 (2.9%) patients, respectively. Eleven (7.9%) individuals had no underlying medical condition and 85 (61.2%) patients exhibited immunosuppression consisting in HIV-infection ($n = 39$), hematopoietic stem cell transplantation (HSCT; $n = 23$), solid organ transplantation (SOT; $n = 5$), hemopathy ($n = 10$), solid cancer ($n = 3$), and immune disorder ($n = 5$). Data concerning immune status were missing for 43 (30.9%) patients. Overall, 200 (94.8%) clinical samples were received from France (metropolitan France, France overseas departments and territories) and 11 (5.2%) from other Europe countries (Switzerland, Belgium, Czech Republic). Samples included 169 (80.1%) mucocutaneous lesions, with oro-facial ($n = 46$) and anogenital ($n = 76$) vesicles, skin swabs ($n = 30$), and keratitis lesions ($n = 17$), 8 (3.8%) cerebrospinal fluids, 5 (2.4%) bronchoalveolar lavage fluids, and 5 (2.4%) blood samples. Collection sites were not itemized for 24 (11.4%) of the clinical samples. Antiviral treatment information was unavailable for 39 (28.1%) patients for whom clinical samples were sent mainly by outside physicians. For the remaining patients, antiviral treatments consisted of intravenous ACV and/or oral VACV ($n = 97$), FOS ($n = 21$), ganciclovir (GCV) ($n = 3$), famciclovir (FCV) ($n = 1$) and cidofovir (CDV) ($n = 1$). Several patients were given successive-line regimens or bitherapy in case of antiviral resistance (Table 1).

Table 1
Characteristics of the study population.

Characteristics	Patients ($n = 139$)
Median age (years), [range]	47 [3–86]
Gender	
Male	73
Female	58
Non specified	8
Demographic data	
Paris and surroundings	93
Provincial town	36
French overseas	3
European countries	7
Immunosuppression	
HIV infection	39
HSCT	23
SOT	5
Hemopathy	10
Solid cancer	3
Immune disorder	5
None	11
Non specified	43
Samples[a]	
Mucocutaneous lesions	169
CSF	8
BAL	5
Blood	5
Non specified	24
Antiviral treatment[b]	
Acyclovir/valacyclovir	97
Foscarnet	21
Ganciclovir	3
Famciclovir	1
Cidofovir	1
Non specified	39

BAL: bronchoalveolar lavage fluid; CSF: cerebrospinal fluid; HIV: human immunodeficiency virus; HSCT: hematopoietic stem cells transplantation; SOT: solid organ transplantation.

[a] For some patients, sequential HSV-positive samples were tested by antiviral resistance testing assay in order to optimize antiviral treatment.

[b] Some patients received several lines of antiviral treatment or multitherapy during HSV infections.