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Resistance of herpes simplex viruses to acyclovir: An update from a ten-year survey in France

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ABSTRACT

The widespread use of acyclovir (ACV) and the increasing number of immunocompromised patients have raised concern about an increase in ACV-resistant herpes simplex virus (HSV). ACV resistance has traditionally been a major concern for immunocompromised patients with a frequency reported between 2.5% and 10%. The aim of this study was to reassess the status of HSV resistance to ACV in immunocompetent and immunocompromised patients over a ten year period, between 2002 and 2011. This was done by retrospectively following 1425 patients. In immunocompetent patients, prevalence of resistance did not exceed 0.5% during the study period; whereas in immunocompromised patients, a significant increase was observed, rising from 3.8% between 2002 and 2006 (7/182 patients) to 15.7% between 2007 and 2011 (28/178) ($p = 0.0001$). This sharp rise in resistance may largely be represented by allogeneic hematopoietic stem cell transplant patients, in which the prevalence of ACV resistance rose similarly from 14.3% (4/28) between 2002 and 2006 to 46.5% (26/56) between 2007 and 2011 ($p = 0.005$). No increase in ACV resistance was detected in association with other types of immune deficiencies. Genotypic characterization of HSV *UL23* thymidine kinase and *UL30* DNA polymerase genes revealed 11 and 7 previously unreported substitutions, respectively. These substitutions may be related to potential polymorphisms, drug resistance, or other mutations of unclear significance.

1. Introduction

Repeated and long-term acyclovir (ACV) use associated with clinical and immunological features may lead to the development of herpes simplex virus (HSV) resistant to this drug. HSV resistance to ACV is primarily a concern for immunocompromised patients whereas the prevalence remains low in immunocompetent patients. Since the commercialisation of ACV in the 1980s, resistance to this drug in immunocompetent patients has been estimated at 0.5% (Christophers et al., 1998; Fife et al., 1994; Nugier et al., 1992). After twenty years of use, the prevalence did not increase (Danve-Szatanek et al., 2004; Stranska et al., 2005). Nevertheless, the recurrent and widespread use of ACV and its prodrug Val-ACV, including FDA-approved short-course and high dose regimens to treat HSV recurrence, may be contributing factors towards the emergence of resistance (Cunningham et al., 2012). One recent Chinese study reported an unexpectedly high prevalence of ACV-resistant HSV of 4% in immunocompetent children with oral herpetic lesions (Wang et al., 2011). Further, some cases of recurrent herpetic keratitis have been found to be associated with ACV-resistant virus with a prevalence of 6.4% in immunocompetent patients (Duan et al., 2009). Additionally, immunocompetent patients with genital herpes have a higher prevalence of ACV-resistant HSV (Kriesel et al., 2005). With regards to immunocompromised patients, the prevalence of ACV-resistant HSV is largely higher and varies between 3.5% and 10%, depending on the type of immunosuppression. HSV resistant to ACV can induce large,

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extensive and ulcerative cutaneous infections, but also esophagitis, pneumonia and encephalitis, which can be fatal without clinical improvement. Among immunocompromised patients, the prevalence of resistance was reported between 3.5% and 7% in HIV patients, 2.5% and 10% in solid organ transplant patients, and with the greatest prevalence witnessed at 4.1% and 10.9% in hematopoietic stem cell transplant (HSCT) patients (Danve-Szatanek et al., 2004; Piret and Boivin, 2011). The prevalence has even been observed as high as 25% in allogeneic HSCT patients (Chakrabarti et al., 2000; Morfin et al., 2004).

95% of resistant cases are attributed to mutations on the *UL23* gene encoding for thymidine kinase and 5% are attributed to mutations on the *UL30* gene encoding for viral DNA polymerase (Piret and Boivin, 2011, 2014). Antiviral drug resistance screening using genotypic techniques are an efficient approach to make prompt virological detection to adapt antiviral treatment. Nevertheless, genotypic characterization of *UL23* and *UL30* genes have revealed many natural polymorphisms in both genes, which can complicate the interpretation of genetic sequences, particularly when detected substitutions have not been previously described or characterized (Burrel et al., 2010).

The objectives of the present study were: (i) to reassess the frequency of ACV resistance in immunocompetent and immunocompromised patients from a ten-year survey; (ii) to describe new potential polymorphisms and resistance mutations, in the *UL23* and *UL30* genes, based on 22 ACV-sensitive and 40 ACV-resistant HSV.

2. Materials and methods

2.1. Patients and specimens

In this single-centre study, during the 2002–2011 period, 1529 samples from 1425 patients had tested HSV-positive, by culture on MRC-5 cells. The University-Hospital of Hospices Civils of Lyon is the regional reference centre of 5500 beds and serves a population of 1.7 million. Some patients were sampled more than once but each sample was considered as a distinct episode of herpetic infection. 70% of patients were adults (1064 clinical samples were obtained from 1005 adults, median age of 50 (range: 18–75)) and 30% were children (465 clinical samples were obtained from 420 children, median age of 6.5 (range: 1–17)). HSV1 was detected in 1337 clinical samples (429 oropharyngeal, 191 nasopharyngeal, 200 cutaneous, 252 bronchoalveolar lavages, 185 genital and 80 ocular samples) and HSV2 in 192 clinical samples (116 genital, 2 nasopharyngeal, 70 cutaneous, 1 bronchoalveolar lavage, and 3 ocular samples) by specific immune fluorescence. CSF and aqueous humor were excluded as they could not be cultured. In the 1064 clinical adult samples, one was from a patient whose immune status was unavailable, 703 were from immunocompetent patients, and 360 were from immunosuppressed patients including 90 hematopoietic stem cell transplants (HSCT) (87 allogeneic and 3 autologous), 37 HIV, 68 haemopathies, 37 non-haemopathy malignancies, 94 solid organ transplants (SOT), and 34 other sources of immunosuppression. From the 465 children samples, 324 were from immunocompetent children and 141 were from immunosuppressed children including 30 allogeneic HSCT, 36 haemopathies, 12 non-haemopathy malignancies, 38 SOT, 4 HIV and 21 other sources of immunosuppression.

2.2. Phenotypic diagnosis of antiviral resistance

Screening for antiviral resistance was systematically performed on the 1529 HSV-positive culture using a neutral red dye-uptake assay to determine its sensitivity to ACV (GlaxoWellcome, France) and FOS (Astra, France), as previously described (Langlois et al., 1986). The effective concentration 50 (EC_{50}) cut-off values for resistance to ACV were 6.5 μM for HSV1 and 13.5 μM for HSV2, and the EC_{50} cut-off value for resistance to FOS was 350 μM for both HSV1 and HSV2 (Nugier et al., 1992).

2.3. Statistical analysis

Student's *t*-test and Person χ^2-test were used to assess intergroup differences between the two timelines 2002–2006 *versus* 2007–2011. The division of the 10-year study period was made with regards to resistance evolution. Statistical analyses were performed on EpiInfo software (V 3.5.1 CDC). Odds ratio (OR) and 95% confidence interval (CI) were calculated to determine the likelihood of detecting ACV-resistant HSV during the two periods. A test was significant when the *p* value was less than 0.05.

2.4. Genotypic analysis of antiviral resistance

Genotypic analysis of ACV-resistant HSV1 and HSV2 was performed directly on clinical samples by sequencing the *UL23* and *UL30* genes, which encodes for thymidine kinase and DNA polymerase, respectively, as previously described (Burrel et al., 2010; Frobert et al., 2008). When a sensitive HSV was previously isolated from a patient harboring a resistant virus, the *UL23* gene of the sensitive strain was also sequenced. Nucleotide sequences were compared with reference strains SC16 (HSV1) and 333 (HSV2) (GenBank accession numbers X03764 and V00466, respectively) using SeqmanII software (DNAStar Inc.).

3. Results

3.1. Frequency of ACV-resistant HSV over 2002–2011

3.1.1. Adults

One case of ACV-resistant HSV was detected in an immunocompetent patient (0.14%) and another case of resistance was detected in a patient whose immune status was unavailable (Table 1). Among 360 immunocompromised patients, 35 cases of resistance were detected (9.7%) with 30 cases from allogeneic HSCT patients (30/87, 34.5%), 3 cases from HIV patients (3/37, 8.1%), 1 case from a patient with haemopathy (1/68, 1.5%) and 1 case from a SOT patient (1/94, 1.1%).

3.1.2. Children

In immunocompetent children, no cases of resistance were detected; but in immunocompromised children, 7 cases were detected (7/141, 5%) with 5 cases coming from allogeneic HSCT patients (5/30, 16.7%), 1 case from a child with haemopathy (1/36, 2.8%) and 1 case from a SOT patient (1/38, 2.6%).

3.2. Resistance evolution

3.2.1. Adults

Over the 2002–2011 period, the frequency of ACV resistance has increased from 1.4% in 2002 to 5.2% in 2011, while the number of clinical samples testing HSV-positive remained relatively consistent year over year (Fig. 1). A significant increase was observed in 2007, prompting the division of the study period to be made between 2002–2006 *versus* 2007–2011 (Fig. 1). The overall prevalence of ACV-resistant HSV in adults increased significantly between 2002–2006 and 2007–2011, from 1.4% to 6% (OR = 4.22; $p = 0.00006$) (Table 1). In immunocompetent adults, the prevalence of resistance remained under 0.5%, throughout the years ($p = 0.59$) (Table 1). In immunocompromised patients, a significant increase