Journal of Clinical Virology 32 (2005) 7–18

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Survey of acyclovir-resistant herpes simplex virus in the Netherlands: prevalence and characterization

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Received in revised form 27 March 2004; accepted 10 April 2004

Abstract

Background: Widespread and frequent use of acyclovir (ACV) for treatment, suppressive therapy and prophylaxis of herpes simplex virus (HSV) infections and its over the counter availability may be associated with emergence of HSV resistance. *Objectives:* To determine the prevalence of ACV-resistant HSV isolates in different patient groups between 1999 and 2002 in the Netherlands. *Study design:* A total of 542 isolates, 410 HSV-1 and 132 HSV-2, from 496 patients were screened for reduced susceptibility to ACV. A newly developed ELVIRA HSV screening assay was used that allowed a high throughput screening. The genotypic analysis of the HSV thymidine kinase gene was performed to identify resistance-associated mutations. *Results:* Thirteen isolates, 8 HSV-1 and 5 HSV-2, from 10 patients (2%) were found resistant to ACV. A single ACV-resistant strain was identified among isolates from 368 immunocompetent patients (0.27%; 95% confidence interval [CI], 0.007%–1.5%), whereas in nine isolates from 128 immunocompromised patients resistant HSV was identified (7%; 95% CI, 3.26%–12.93%). The highest frequency of ACV-resistant HSV was associated with bone marrow transplantation: four patients out of 28 (14.3%) shed resistant virus. In addition, resistant virus was obtained from two HIV-positive patients, one patient with a hematological malignancy and two patients on immunosuppressive drugs. Further testing showed that none of the isolates was resistant to foscarnet. Several new mutations were identified in the thymidine kinase gene of these resistant isolates, and their effect on ACV-resistance is discussed. *Conclusions:* Our study shows that the prevalence of ACV resistance is low in immunocompetent patients (0.27%), whereas ACV-resistant HSV infections occur relatively frequently in immunocompromised patients (7%; $P < 0.0001$). This emphasizes the need for drug susceptibility monitoring of HSV infections in immunocompromised patients with persisting infections despite antiviral therapy.

Keywords: Acyclovir-resistant herpes simplex virus; Acyclovir; HSV thymidine kinase gene

1. Introduction

For more than 20 years, acyclovir (ACV) has been the drug of choice for prophylaxis and treatment of herpes simplex virus (HSV) infections. Its use is indicated for treatment of primary and recurrent genital HSV infection as well as for chronic suppressive treatment of genital herpes, and as a first line treatment for HSV encephalitis, where timely administration of ACV is needed to prevent often fatal outcome (Whitley and Lakeman, 1995). Oral or intravenous ACV is frequently used for prophylaxis and treatment of HSV infections in immunocompromised patients. In the context of severe immunosuppression, as established for example during hematopoietic stem cell transplantation (HSCT), ACV prophylaxis dramatically decreases HSV reactivation in seropositive HSCT recipients, in whom the risk of chronic, severe and sometimes fatal HSV infections is high (Bergmann et al., 1995; Chen et al., 2001; Dignani et al., 2002; Shepp et al., 1987; Wood, 1998). Finally, topical ACV and penciclovir (PCV) formulations are available

Abbreviations: HSV, herpes simplex virus; CMV, cytomegalovirus; ACV, acyclovir; PFA, foscarnet; GCV, ganciclovir; TK, thymidine kinase; DNA pol, DNA polymerase; HSCT, hematopoietic stem cell transplantation; STD, sexually transmitted disease; HFF, human foreskin fibroblasts; CV, coefficient of variation

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doi:10.1016/j.jcv.2004.04.002

in most countries as an over the counter drug for management of recurrent herpes labialis.

ACV, a nucleoside analogue, requires three phosphorylation steps to achieve an antiviral effect by competitive inhibition of viral DNA polymerase activity. The initial phosphorylation step is carried out by the viral thymidine kinase (TK), and the two subsequent ones by cellular kinases. Antiviral resistance is mostly conferred by mutations in the TK gene (nucleotide additions, deletions or substitutions) (Bestman-Smith et al., 2001) and, to a much lesser extent by mutations in the viral DNA polymerase (DNA pol) gene (Larder and Darby, 1985, 1986).

Since the introduction of ACV, concerns have been raised, that long-term treatment, prophylaxis and suppressive use may result in the development of resistance. ACV-resistant viruses have been found in clinical isolates, which were never exposed to ACV, and ACV-resistant TK mutants of HSV can be readily selected in vitro (Parris and Harrington, 1982; Sarisky et al., 2001). These in vitro observations were confirmed by an increased frequency of isolation of drug-resistant HSV viruses from ACV-treated immunocompromised patients since the early 1990s (Chen et al., 2001). Studies reporting on the prevalence of ACV-resistant HSV have been recently reviewed (Bacon et al., 2003). Combined with the results from the most recent studies by Danve-Szatanek et al. (Danve-Szatanek et al., 2004) and Reyes et al. (Reyes et al., 2003) it can be concluded that the prevalence of HSV infections with reduced susceptibility to ACV in immunocompromised patients varies from 3.5% and 7.1% (Bacon et al., 2003; Christophers et al., 1998; Englund et al., 1990; Nugier et al., 1992). The highest prevalence rates are reported for recipients of HSCT, with a range from 4.1% to 10.9% (Chen et al., 2001; Danve-Szatanek et al., 2004; Wade et al., 1983; Williamson et al., 1999). A recent report described an even higher frequency (36%) of ACV resistance (Langston et al., 2002). Similarly, the prevalence in HIV-positive patients ranges from 3.5% to 7% (Englund et al., 1990; Danve-Szatanek et al., 2004; Reyes et al., 2003) and in solid organ transplant (SOT) recipients from 2.5% to 10% (Christophers et al., 1998; Danve-Szatanek et al., 2004). The numbers of patients in the SOT group, however, are usually low.

In contrast, ACV-resistant isolates have been reported infrequently in immunocompetent subjects (Bacon et al., 2003). This is probably due to the low pathogenic potential of the resistant virus variants and the presence of effective immune response, which results in rapid clearance of the virus (Coen et al., 1989). A low prevalence of resistant HSV in immunocompetent patients was reported in extensive screening surveys performed in the UK (0.7%) and the USA (0.3%) between 1980 and 1992 (Collins and Ellis, 1993). No increase in the prevalence of resistance has been observed since then (range from 0.1% to 0.7%) (Bacon et al., 2002; Bacon et al., 2003; Boon et al., 2000; Christophers et al., 1998; Danve-Szatanek et al., 2004; Reyes et al., 2003). These studies, performed in the UK, USA and France, also included patients on chronic suppressive therapy for genital herpes as well as the general population using ACV/PCV topical preparations for herpes labialis.

Our study aimed at obtaining data on the prevalence of ACV-resistant HSV among isolates collected from different patient groups between 1999 and 2002 in The Netherlands, with a focus on determination of the prevalence rates in both the immunocompetent and immunocompromised patient populations. For both the general population as well as for specific patient groups, this study set baseline prevalence estimates for future national surveillance studies. In addition, our study also included a detailed phenotypic and genotypic characterization of resistant clinical isolates identified in the survey.

2. Methods

2.1. Study participants

HSV isolates were obtained from major clinical virological laboratories that provide routine diagnostic service for university and regional hospitals, STD clinics as well as general practitioners' practices in The Netherlands.

2.2. Clinical specimens

HSV-positive specimens, either culture isolates or original materials, were obtained from the participating laboratories. The laboratories were asked to provide a random collection of HSV-positive specimens/isolates obtained between 1999 and 2002. No other selection criteria were applied. Isolates were coded and susceptibility testing was performed without prior clinical information on the patients' specimens in order to avoid bias in the susceptibility test results. Clinical data were coupled to the results of susceptibility testing only when a final test result was obtained. Efforts were made to obtain information on the patients' diagnosis, immunostatus, clinical manifestations of HSV disease and previous use of ACV. Therefore, participating laboratories were requested to complete a basic questionnaire. Additional clinical information was collected from the medical records of specific patients of interest (i.e. Patients with resistant HSV isolates). However, in several cases, complete and detailed information could not be obtained.

HSV isolation and serotyping were performed in the collaborating laboratories using their routine diagnostic procedures. Specimens included skin, oro-facial and ano-genital specimens obtained from vesicles or ulcerative lesions, as well as ocular, throat and lower respiratory tract specimens and biopsies. For susceptibility screening, small scale virus stocks were prepared on human foreskin fibroblasts (HFF) from all obtained specimens and stored at $-70\,^{\circ}C$. The number of in vitro passages for individual HSV-positive specimens was limited to two.