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Resistance testing of clinical varicella-zoster virus strains

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ABSTRACT

Acyclovir resistance of varicella-zoster virus (VZV) has been reported in rare cases of immunocompromised patients. In this study, the natural polymorphism of the thymidine kinase (TK) and DNA polymerase (pol) genes was examined in 51 clinical VZV isolates sensitive to acyclovir (ACV). In addition, 16 VZV strains with clinical resistance to ACV were analyzed. None of the ACV-sensitive strains of the clades 1, 3 and 5 showed gene polymorphism of the TK. By contrast, the DNA pol gene exhibited polymorphism-related substitutions as a function of the VZV clade. The novel substitutions M286I, E824Q, R984H and H1089Y were detected in strains of clades 3 and 5. In the TK gene of 7 VZV strains with clinical ACV resistance, the novel substitutions L73I, A163stop, W225R, T256M, N334stop and the deletion of nucleotides 19–223 were found to be associated most likely with resistance. In one strain showing the substitution W225R, ACV resistance could be confirmed by the viral phenotype. In the DNA pol gene, the novel amino acid substitutions T237K and A955T could be detected, but their significance remains unclear. In conclusion, the characterization of resistance using genetic analysis of the TK and DNA pol genes has to be considered the method of choice for the determination of VZV resistance to antiviral drugs. In a considerable number of patients with clinical ACV-resistant VZV infections, resistance cannot be verified by virological methods.

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1. Introduction

Varicella-zoster virus (VZV) is a member of the Herpesviridae family and is divided into the five major clades 1–5 (Breuer et al., 2010) showing a distinctive geographic distribution in temperate versus tropical regions (Loparev et al., 2007; Quinlivan et al., 2002). VZV causes two distinct diseases. During childhood, primary infection results in typical signs of varicella, and zoster is caused by endogenous reactivation after the virus has established lifelong latency. The occurrence of zoster is thought to be associated with waning VZV-specific T-cell-mediated immunity (Gershon et al., 1997), e.g. in the elderly or in patients with immunodeficiency. While complications of varicella are rarely observed in immunocompetent infants, patients with impaired cellular immune function, e.g. patients with oncological diseases, organ or bone marrow transplantation, autoimmunopathies, congenital immune defects or persons infected with the human immunodeficiency virus, have a special risk of severe courses of varicella (Arvin, 1999). Varicella pneumonia has been considered the most important complication in pregnant women. Furthermore, maternal infection during the first two trimesters or near term can be associated with a substantial risk of intrauterine infection or neonatal varicella (Sauerbrei and Wutzler, 2007). Zoster is often complicated by pain termed as post herpetic neuralgia if the pain persists after the rash healed (Gilden et al., 2009). Other important complications include neurological manifestations, hemorrhagic and necrotic alterations of the skin, bacterial super-infections and eye or ear involvement. In immunocompromised patients, zoster is associated with significant morbidity and mortality due to disseminated and chronic infections (Au et al., 2003; Gnann, 2002).

The current drug of choice for the antiviral treatment of VZV infections in patients at risk is the nucleoside analogue acyclovir (ACV) (De Clercq, 2004). Because of the low bioavailability, the drug has to be administered intravenously for 7–10 days (Gross et al., 2003). For oral treatment of VZV infections, especially of zoster, valyciclovir, the prodrug form of ACV, famciclovir, the prodrug of penciclovir (PCV) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU, brivudin) are available. The general mode of action of these nucleoside analogues is through inhibition of viral DNA polymerase (pol) by acting as competitive inhibitors and/or DNA chain terminators. This action requires three intracellular phosphorylation steps to convert the nucleoside analogues into their mono-, di- and triphosphates. The viral thymidine kinase (TK), which acts as thymidine and thymidylate kinase, is involved in the first phosphorylation step of ACV and PCV and equally in the second phosphorylation step of BVDU. Acyclovir resistance has to be assumed if clinical findings improve only slowly or not at all (Gross et al.,

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2003). In such cases, another treatment option would be intravenous foscarnet (FOS). This pyrophosphate analogue acts directly on the viral DNA pol by impeding pyrophosphate release from deoxynucleotides during DNA syntheses (Chrisp and Clissold, 1991). Likewise, the acyclic nucleoside phosphonate cidofovir (CDV) has been shown to be active against VZV and a broad range of DNA viruses (De Clercq, 2004). Following intracellular phosphorylation to the diphosphate form, CDV acts as chain terminator.

Acyclovir and/or FOS resistance of VZV has only been reported in rare cases of immunocompromised patients, such as in persons developing the acquired immune deficiency syndrome (AIDS) (Boivin et al., 1994; Bryan et al., 2008; Crassard et al., 2000; Fillet et al., 1998; Hatchette et al., 2008; Jacobson et al., 1990; Linnemann et al., 1990; Lyall et al., 1994; Morfin et al., 1999; Pahwa et al., 1988; Safrin et al., 1991; Saint-Léger et al., 2001; Snoeck et al., 1994; Talarico et al., 1993; Visse et al., 1998; Wunderli et al., 1996). Similarly to the herpes simplex virus (HSV), resistance to ACV is associated with deficient TK activity (Morfin et al., 1999) or, less often, with an alteration of substrate specificity (Boivin et al., 1994). Acyclovir or FOS resistance conferred by mutations in the DNA pol gene has also been described (Fillet et al., 1994; Pahwa et al., 1988).

The aim of this study was to characterize the natural polymorphism of both the TK and DNA pol genes of 51 clinical ACV-sensitive VZV isolates obtained from patients with varicella or zoster. In addition, 16 VZV strains with clinical resistance to ACV were analyzed genotypically for resistance. In 3 out of these strains, genotypic findings could be compared with phenotypic features.

2. Materials and methods

2.1. Viral strains and cell cultures

In this study, 51 ACV-sensitive VZV isolates from 29 patients with varicella and 22 patients with zoster were included. From the patients with varicella, aged between 1 and 37 years (mean 7.2 years), 17 were male and 12 female. The age of zoster patients, 11 were male and 11 were female, was between 3 and 87 years (mean 47.3 years). Strains were isolated in human embryonic lung fibroblasts (HELF) (Sauerbrei et al., 1999) between 2000 and 2008 from vesicle fluid and in one case from pharyngeal tissue after the patients' specimens were sent to the German reference laboratory for HSV and VZV for diagnosing VZV infection. There was no information about any antiviral therapy in these patients. No patient had received varicella vaccination. The reference strains parental Oka (pOka) and vaccine Oka (vOka), kindly provided by the Institute of Virology, Charité Medical School, Berlin, Germany, were used as controls. Testing of resistance phenotype (method described below) revealed inhibitory concentrations 50% (IC_{50}) of ACV between 0.4 and 1.3 μg/ml (1.8–5.8 mM). Considering the cut-off value for resistance (see also 2.3.), all strains were ACV-sensitive. The viral strains were genotyped using the scattered single nucleotide polymorphism (SNP) method on the basis of sequencing open reading frames 1, 21, 22, 37, 50, 54 and 60 (Sauerbrei et al., 2008) and classified into the major clades described recently (Breuer et al., 2010).

Furthermore, the study included 16 VZV strains that were obtained from 15 patients with zoster and one patient with VZV encephalitis for resistance testing because of clinical resistance against ACV. This means, there was no clinical improvement under administration of ACV for at least 10 days (Balfour et al., 1994; Safrin et al., 1991). However, detailed information about the administration of ACV including any alternative treatment with FOS was not available. Patients' data and clinical information are summarized in Table 1. There were 6 female and 8 male patients and their age was between 6 and 66 years (mean 38.4 years). Age and gender were not known in 2 patients. Strains were obtained from vesicle fluid in case of zoster and from cerebrospinal fluid in case of encephalitis. All clinical samples were submitted to the German reference laboratory for HSV and VZV to verify ACV resistance by laboratory methods. VZV strains could be isolated in HELF from the vesicle fluids of 3 patients with zoster (No. 2, 3 and 5), but in the remaining cases cell culture isolation of viruses was not successful.

Table 1
Clinical data of patients.

No. of viral strain	Patient		Clinical data (diagnosis)
	Age (years)	Gender	
1	n.a.	n.a.	Recurrent generalized zoster after therapy with ACV, NHL
2	57	m	Persistent zoster under therapy with valacyclovir, TCL
3	36	f	Persistent zoster under therapy with ACV after BMT
4	n.a.	n.a.	Persistent zoster under therapy with ACV, CLL
5	14	f	Persistent zoster trigeminus under therapy with ACV, AML
6	6	m	Persistent zoster under therapy with ACV, IS
7	66	f	Persistent zoster under therapy with ACV, IS
8	49	f	Persistent zoster under therapy with ACV, IS
9	18	f	Zoster under chemoprophylaxis with ACV, IS
10	71	m	Persistent zoster under therapy with ACV
11	11	m	Zoster under chemoprophylaxis with ACV after SCT
12	64	m	Persistent zoster under therapy with ACV after SCT
13	7	m	Persistent zoster under therapy with ACV, IS
14	31	m	Persistent VZV encephalitis under ACV, IS
15	43	m	Persistent VZV pneumonia under therapy with ACV, HCL
16	64	f	Persistent zoster under therapy with ACV, IS

ACV – acyclovir, AML – acute myeloid leukaemia, BMT – bone marrow transplantation, CLL – chronic lymphocytic leukaemia, HCL – hairy cell leukaemia, IS – immunosuppression, NHL – Non-Hodgkin's lymphoma, SCT – stem cell transplantation, TCL – T-cell lymphoma, f – female, m – male, n.a. – not available.

2.2. Antiviral test compounds

The following antiviral compounds were used for the phenotypic characterization of resistance to VZV: acyclovir (ACV: GlaxoSmithKline, Uxbridge, UK), brivudin (BVDU; Berlin-Chemie AG, Berlin, Germany), penciclovir (PCV; GlaxoSmithKline, Uxbridge, UK), foscarnet (FOS; AstraZeneca, Wilmslow, UK), and cidofovir (CDV; Vistide®, Pharmacia and Upjohn, Luxembourg).

2.3. Phenotypic characterization of resistance

VZV strains were grown and titrated in human Caucasian fetal lung fibroblasts of the cell line Wi 38 (European Collection of Cell Cultures, Salisbury, UK) using the method described previously (Sauerbrei et al., 2007). Antiviral testing was performed in 48-well flat-bottomed microtitre plates by means of plaque reduction assay. First, 200 μl of Wi 38 cell suspension per well were seeded at a density of 2×10^5 ml^{-1}. After addition of antiviral compounds at a final half log dilution over a range between 0.0625 and 8 μg ml^{-1} (ACV, PCV, CDV), between 0.0001 and 8 μg ml^{-1}

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