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Case Report

Reactivation of Acyclovir-Resistant Thymidine Kinase-Deficient Herpes Simplex Virus Harbouring Single Base Insertion Within a 7 Gs Homopolymer Repeat of the Thymidine Kinase Gene

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HSV infections are treated efficiently and prevented by acyclovir, although resistant strains have been reported. Resistance to acyclovir involves mainly mutations in the viral gene encoding thymidine kinase; mutations may lead to an altered or, more frequently, deficient TK. These acyclovir-resistant TK deficient strains are not able to reactivate from a latent infection in an experimental model, compared to TK positive strains. A case is reported of a bone marrow transplant child who developed HSV infection at 11 days post-transplantation. Acyclovir-resistant HSV 1 was isolated on day 19 post-transplantation. The patient was cured of his infection. A resistant virus was detected 20 months later that harboured the same TK gene mutation as the first resistant virus. This mutation is an insertion of one guanine in a homopolymer repeat of seven guanines located at codon 146 of TK. It has previously been reported and associated with the expression of a deficient TK activity and the ability to reactivate in mice. These results corroborate the clinical relevance of this mutation, which is associated with acyclovir-resistant recurrent infections in humans. ***J. Med. Virol. 62: 247–250, 2000.***

KEY WORDS: bone marrow transplantation; herpes simplex virus; thymidine kinase-deficient; reactivation

INTRODUCTION

Herpes simplex virus (HSV) infections occur frequently in immunocompromised patients. Acyclovir is used widely and the emergence of resistant strains has been reported mainly in AIDS patients but also in bone marrow transplant recipients [Darville et al., 1998; Morfin et al., 2000]. HSV resistance to acyclovir has been associated with mutations occurring on either the viral thymidine kinase (TK) gene or the viral DNA polymerase gene. Mutations affecting viral TK activity may be associated with a deficient activity (TK-deficient virus) or with an alteration of substrate specificity (TK-altered virus). Most cases of clinical resistance of HSV to acyclovir have been associated with TK-deficient virus. Numerous mutations occurring in the TK gene have been described in acyclovir-resistant clinical HSV strains, but some are reported repeatedly. A substitution of arginine 176 of HSV 1 and 177 of HSV 2 has been found in both clinical and laboratory derived resistant strains [Darby et al., 1986; Nugier et al., 1991; Kost et al., 1993; Gaudreau et al., 1998]. A modification of the amino acid 336 has also been found in clinical strains as well as laboratory-selected resistant viruses [Darby et al., 1986; Rechtin et al., 1995; Gaudreau et al., 1998]. Nucleotide additions or deletions have also been reported often; they are responsible for a frameshift and the synthesis of a truncated, non functional TK [Gaudreau et al., 1998; Kit et al., 1987; Palù et al., 1992]. Most of these insertions or deletions are located in Gs or Cs homopolymer repeats.

Viral pathogenesis of mutant virus depends on resistance phenotype. Conversely to DNA polymerase and TK-altered mutants, TK-deficient HSV are known to have an impaired pathogenecity in animal models; in particular, they fail to reactivate from latency in explanted mouse ganglia: TK activity is not involved in the establishment of a latent infection but is necessary for virus reactivation from latency [Coen et al., 1989].

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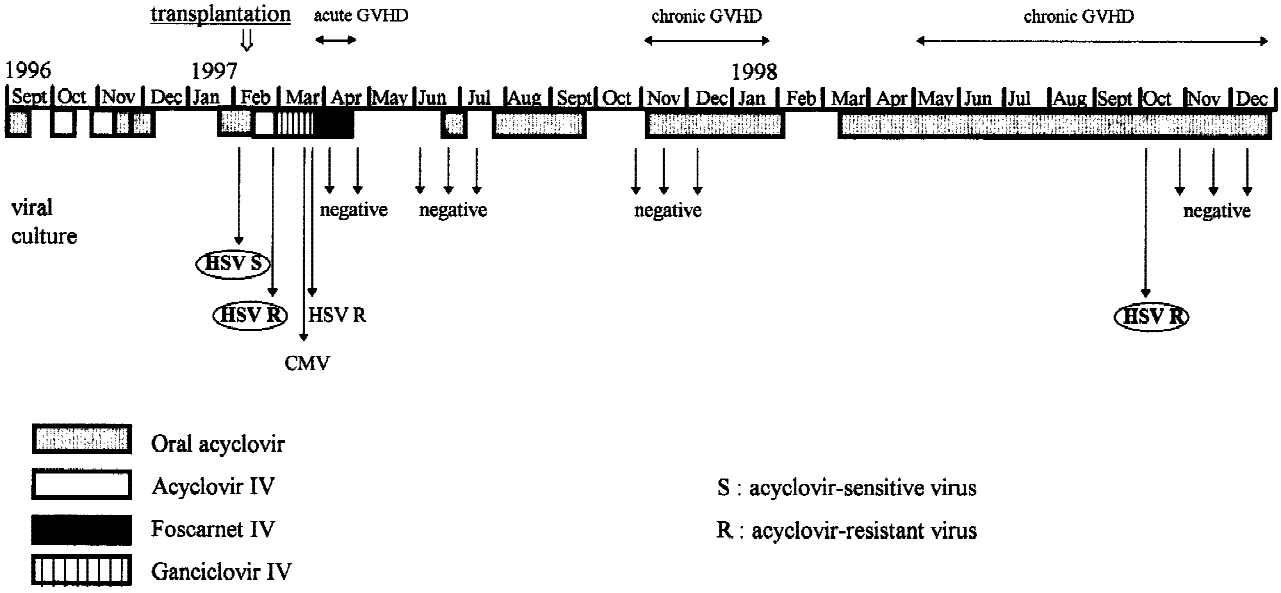

Fig. 1. Patient's clinical history.

After isolation of an acyclovir-resistant HSV, reactivations are usually associated with the original, TK-positive, acyclovir-sensitive strain.

The case is reported of a bone marrow transplant recipient who developed recurrent HSV 1 infections due to an ACV-resistant virus harbouring a mutation within a 7 Gs homopolymer repeat of its TK gene. Twenty months after a complete healing of the patient's infection, an acyclovir-resistant virus presenting the same mutation was isolated. This case corroborates the clinical relevance of specific TK-deficient acyclovir-resistant mutants that can cause recurrent infections.

CASE REPORT

A male patient, born in 1986, was referred with high-risk acute lymphoblastic leukaemia [L1, immunophenotype Pre-B with translocation t(4;11)], which was diagnosed in 1996. The patient underwent transplant during the first complete remission. He received, on February 7, 1997, a bone marrow transplant from an HLA identical unrelated female donor after a conditioning regimen, which included fractionated total body irradiation, VP16 and horse anti-lymphocyte globulins (total dose of 60 mg/kg). Full grafting occurred on day 21 after transplantation. The patient presented with cutaneous acute graft versus host disease (GVHD) grade 1 on April 1, 1997. A favourable evolution was obtained with corticosteroids. From November 1998 until January 1999, and later in May 1999, extensive chronic GVHD re-occurred. He was then treated with corticoids and azathioprin, followed by cyclosporin in June and puvatherapy in September.

To prevent HSV infection, the patient received oral acyclovir (200 mg × 4 per day), beginning 10 days before transplantation. Nevertheless he developed mouth ulcers (grade 1), culture positive for HSV 1 on day 11 post-transplantation (isolate 1, acyclovir-sensitive). Oral treatment was replaced by intravenous acyclovir (500 mg/m^2 × 3 per day). An acyclovir-resistant HSV 1 (isolate 2) was isolated in a broncho-alveolar lavage taken at day 19 post-transplantation. The infection was cured with foscarnet (3.5 g twice a day for 13 days and once a day for 8 days). Meanwhile, a cytomegalovirus was isolated (day 27) and ganciclovir treatment was initiated (10 mg/kg/day for 10 days). In October 1998, 20 months after transplantation, an HSV 1 was again isolated (isolate 3) on a systematic virological survey while the patient was asymptomatic; this virus was resistant to acyclovir. Between isolates 2 and 3, 12 attempts were made to isolate HSV that all remained negative. The clinical history is summarised in Figure 1.

VIROLOGICAL STUDIES

Isolates 1 (18/02/97) and 3 (06/10/98) were isolated from throat samples, whereas isolate 2 (26/02/97) was isolated from broncho-alveolar lavage (BAL). Viruses were cultivated on Vero cells. Susceptibilities to acyclovir (GlaxoWellcome, Marly-le-Roi, France) and foscarnet (Astra Nanterre, France) were assessed with a dye-uptake assay as previously described [Langlois et al., 1986]. DNA was extracted from infected cells with proteinase K, purified with a standard phenol and chloroform-isoamylic alcohol protocol, and precipitated with ethanol. TK gene was amplified with two overlapping PCR. The first one started 38 nucleotides before the ATG initiation codon and ended at nucleotide 581, using the primers TK1 5′-GGGGATCCTCCCGCACCTCTTTGGC and TK3 3′-CGCAAGCACCGGGAGTACCTAGGGG. The second region started at nucleotide 526 and ended 19 nucleotides after the stop codon,

TABLE I. Phenotypic and Genetic Studies*

Virus isolation date	Biological sample	IC_{50} (μM)		TK gene sequence	
		Acyclovir	Foscarnet	Nucleotide change	Amino acid change
Isolate 1 18/02/97	throat	1.3 - S	157 - S	16: T→G 266:A→G 751: G→T 799: G→T 802: C→A 858: C→A 1100: T→C 1129: A→C	6: Cys→Gly 89: Gln→Arg 251: Gly→Cys 267: Val→Leu 268: Pro→Thr 286: Asp→Glu 367: Met→Thr 376: Asn→His
Isolate 2 26/02/97	BAL	48 - R	125 - S	16: T→G 266: A→G **436: ins G** 751: G→T 799: G→T 802: C→A 858: C→A 1100: T→C 1129: A→C	6: Cys→Gly 89: Gln→Arg **146: frameshift stop codon: 225**
Isolate 3 06/10/98	throat	31 - R	131 - S	16: T→G 266: A→G **436: ins G** 751: G→T 799: G→T 802: C→A 858: C→A 1100: T→C 1129: A→C	6: Cys→Gly 89: Gln→Arg **146: frameshift stop codon: 225**

*IC_{50}: inhibitory concentration 50%; S, sensitive; R, resistant.

using the primers TK 2 5′-GGGGATCCGATACCTTATGGGCAGC and TK 4 3′-TGTGCCTTCCTCTGTTACCTAG GGG.

PCR was performed with the GC rich PCR system® (Roche, Meylan, France) under the following conditions: 5 cycles at 94°C for 20 seconds, 55°C for 30 seconds, and 75°C for 5 minutes, followed by 30 cycles at 94°C for 20 seconds, 55°C for 30 seconds, and 75°C for 1 minutes, with a final extension step at 75°C for 5 minutes. The amplified DNA was purified through a phenol and chloroform-isoamylic alcohol extraction after migration in a low-melting agarose gel. The coding strand of the amplified DNA was sequenced with an automated sequencer (ESGS company, Evry, France). TK gene sequences were compared to that of the ACV-sensitive reference HSV 1 strain KOS [Irmiere et al., 1989].

RESULTS AND DISCUSSION

In the case reported, a child presented with a reactivation due to an acyclovir-resistant virus. Recurrent infections following the emergence of an acyclovir-resistant HSV are usually associated with acyclovir-sensitive virus, although there are a few reports assessing reactivations due to acyclovir-resistant virus, mainly associated with TK-altered virus [Kost et al., 1993]. There is one report dealing with HSV reactivation due to TK-deficient virus: in 1992, Palù et al. notified the isolation of the same acyclovir-resistant TK-deficient virus in an AIDS patient over an 8 month period, which may be relevant to multiple reactivations or chronic excretion of this mutant. Recently, Saijo et al. [1999] reported the isolation of an acyclovir-resistant virus over a 4 year period; these viruses all presented a deletion of one nucleotide that is responsible for a frameshift at codon 355 and the synthesis of a longer TK polypeptide (407 amino acids), resulting in an altered TK activity [Saijo et al., 1998]. Here we describe a reactivation due to a resistant virus that had been already isolated 20 months previously. Numerous negative attempts were made to isolate HSV between these two resistant isolates, thus proving that there was no chronic excretion of the resistant virus but a reactivation from a latent infection.

Phenotypic and genetic characterisation of the three clinical isolates (one sensitive and two resistant) are presented in Table I. Several amino acid substitutions have been detected in the TK gene of the acyclovir-sensitive clinical isolate and the two acyclovir-resistant isolates, suggesting that all three are the same virus. These substitutions have been reported previously in acyclovir-sensitive clinical isolates by Kudo et al. [1998] for codons 6, 89, 251, 267, 268, 286, and 376, and in a laboratory strain by McKnight [1980] for codon 367; they are associated with TK gene polymorphism.

The same TK gene mutation that may account for ACV resistance was detected in the two resistant isolates from our patient. It is an addition of one G in a homopolymer harbouring 7 Gs located at nucleotide 429 to 436 relative to the initiation codon (codon 144 to 146). This addition results in a premature stop codon at 225. Frameshift mutations occurring within homopolymers of Gs or Cs have often been reported in acyclovir-resistant clinical isolates. A deletion or insertion of one

nucleotide in this 7 Gs homopolymer has been reported in 11 HSV isolates resistant to acyclovir; this mutation has always been associated with a TK-deficient phenotype [Gaudreau et al., 1998; Hwang et al., 1994; Sasadeusz et al., 1997; Swetter et al., 1998]. Although it has been shown that these mutants were able to express a low level of TK (1%) due to a net+1 translational recoding event; this frameshift is linked to a structure harbouring G(7)AG [Hwang et al., 1994; Horsburgh et al., 1996]; these viruses prove to be able to reactivate from latent infection of mouse trigeminal ganglia [Hwang et al., 1994].

In the case reported above, we have detected the same nucleotide insertion in both the first resistant virus and the second one, isolated 20 months afterwards, whereas the patient had been cured of his HSV infection. This virus, presenting a nucleotide insertion at codon 146 leading to a deficient-TK, proves to be able to reactivate from a latent infection. However it cannot be excluded that the second acyclovir-resistant virus originates from the parental acyclovir-sensitive strain. The mutation detected is indeed frequent: it has been reported in 11 acyclovir-resistant HSV out of 43 characterised viruses. Accordingly, there is about one chance out of four that the second resistant virus would have been selected from the parental sensitive strain through a mutation on the same nucleotide as the first resistant virus. The likelihood that the second resistant virus originates from the first one is confirmed by the ability of this mutant to reactivate from a latent infection that has been demonstrated in mice.

These results are clinically relevant since the acquisition of such a mutation could be a constant feature with recurrent infections due to ACV-resistant viruses. Moreover, since viruses excreted during recurrences are a major source of contamination, the risk of transmission of these resistant viruses must be investigated.

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