ACYCVIR-RESISTANT CHRONIC VERRUCOUS VACCINE STRAIN VARICELLA IN A PATIENT WITH NEUROBLASTOMA

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Abstract: A 21-month-old girl with neuroblastoma developed chronic verrucous Oka strain varicella-zoster infection during chemotherapy. Virus isolated from the patient demonstrated high-level acyclovir resistance, and its thymidine kinase had no in vitro enzymatic activity. After foscarin therapy, she underwent stem cell transplantation without varicella reactivation. This is only the second reported case of resistant varicella zoster virus caused by Oka strain virus.

Key Words: varicella, Oka, acyclovir, resistance, immunosuppressed, neuroblastoma

Accepted for publication March 20, 2008.

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These studies were supported by Public Health Service contract NOI-AI-00138 (to M.N.P.) from NIH NIAID. Cidofovir was a gift from Merck & Co., Inc. (Whitehouse Station, NJ), and foscarnet was a product of Wyeth-Ayerst (Collegeville, PA). The first two authors contributed equally to this work.

UAB Institutional Review Board approved informed consent was obtained from the patient’s family before compilation of this report. No contributors to the manuscript have any proprietary, financial, professional, or other personal interest of any nature or kind in any product, service, or company that could be construed as influencing the material, position, or review of the manuscript.

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DOI: 10.1097/INF.Ob013e318175d85c

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text; simply type the URL address into any Web browser to access this content. Clickable links to the material are provided in the HTML text and PDF of this article on the Journal’s Web site (www.pidj.com).

The varicella zoster virus vaccine strain Oka (V-Oka) is highly attenuated and can prevent severe disease associated with varicella infection. However, in rare instances it can cause disseminated infections in immunosuppressed patients that require prompt and prolonged therapy.

CASE REPORT

A 21-month-old girl was diagnosed with stage IV neuroblastoma. She had received varicella immunization in her left thigh approximately 1 week before diagnosis and had no other known history of varicella exposure. Her chemotherapy plan consisted of a combination of 4 courses of cyclophosphamide, adriamycin, and vincristine (CAV) and 2 courses of cisplatin and etoposide (CDDP-E) to be followed by autologous stem cell transplant.

Four weeks into chemotherapy, during a period of leukocyte recovery, the patient developed 2 erythematous, umbilicated papules on her right finger and right lower abdomen distant from the immunization site. In the ensuing week, these lesions evolved into vesicular patches, primarily involving her hands, right leg, and abdomen. Serum anti-varicella IgG was positive at this time and polymerase chain reaction (PCR) of lesion scrapings was positive for varicella zoster virus (VZV). Intravenous acyclovir (IV ACV) was started at 60 mg/kg/d and the lesions improved after 1 week of IV therapy. Over the next 2 weeks, therapy was changed to oral ACV therapy at 2 separate times (90 mg/kg/d and later 30 mg/kg/d), but readmission was required for IV therapy within 2 days each time because of worsening lesions and missed oral doses as a result of enesis. Finally, IV ACV (60–70 mg/kg/d) was restarted and continued for 3 additional weeks. During the 5 weeks of IV and oral ACV therapy, the lesions became verrucous and dusky but never healed completely (Fig. 1, http://links.lww.com/A481), and new vesicles continued to erupt intermittently, primarily on her right leg and lower abdomen. VZV was isolated from a newly erupted vesicle, and this isolate exhibited high level resistance in vitro to both ACV and penciclovir (Table 1). Therapy was changed to IV foscarnet (165 mg/kg/d) and continued for a total of 4 months with intravenous immunoglobulin (1 g/kg/dose) given approximately every 2–4 weeks. After 4 weeks of foscarnet therapy, repeated cultures of skin scrapings were culture negative but remained VZV PCR positive.

Eight weeks after starting foscarnet, the patient developed lethargy, fatigue, photophobia, and conjunctivitis. Lumbar puncture revealed leukocytosis (protein 166 mg/dL, 5 WBC per HPF, 92% lymphocytes), and PCR of cerebrospinal fluid (CSF) was positive for VZV, though live virus was never isolated from the CSF. The 6th planned cycle of chemotherapy was withheld, and she received 1 dose of intravenous varicella immunoglobulin (Varizig; Cangene Corporation, Winnipeg, Canada) on an investigational compassionate use basis. Additional doses of Varizig were withheld because of acute renal insufficiency and proteinuria. The patient’s symptoms slowly improved, but cerebrospinal fluid obtained by serial lumbar puncture remained positive for VZV by PCR despite foscarnet therapy for 10 weeks. All virus cultures of the CSF were negative. She exhibited moderate developmental delay neurologically, possibly because of CNS varicella or anesthesia-related respiratory arrest that occurred early in therapy.

After completing induction chemotherapy and consolidative radiotherapy, she remained in remission on 13-cis retinoic acid for TABLE 1. Sensitivity of the Oka and Isolate 6/08/2 to Selected Antiviral Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>Fold-Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganciclovir</td>
<td>11 ± 6</td>
<td>47 ± 13.2</td>
</tr>
<tr>
<td>Cidofovir</td>
<td>0.50 ± 0.01</td>
<td>0.7 ± 0.14</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>6.3 ± 2.2</td>
<td>&gt;100 ± 0</td>
</tr>
<tr>
<td>Penciclovir</td>
<td>15 ± 0.07</td>
<td>&gt;100 ± 0</td>
</tr>
<tr>
<td>Foscarnet</td>
<td>58 ± 1.3</td>
<td>60 ± 8.4</td>
</tr>
</tbody>
</table>

*EC<sub>50</sub> values are the drug concentrations required to reduce plaque number by 50% and are given in units of micromolar with the standard deviations shown.
3 months, but neuroblastoma recurred. She underwent reinduction chemotherapy with ifosfamide, etoposide, cyclophosphamide, and topotecan with good response and ultimately had a successful autologous stem cell transplant utilizing VZV PCR negative peripheral blood stem cells. She remained in good health until 6 months post-transplantation when neuroblastoma again relapsed. After a trial of palliative therapy, the child died from complications of her disease. She never demonstrated clinical evidence of varicella re-infection after transplant.

MATERIALS AND METHODS

**Virus Purification and Characterization.** Varicella-zoster strain Oka was obtained from American Type Culture Collection (Manassas, VA) and passaged as described previously. The clinical isolate (6/06/2) was obtained from a swab of unrepaired lesions from the patient. This isolate was placed in 25 cm² flasks with freshly trypsinized primary human foreskin fibroblast cells. Infected cells were passaged and expanded to produce virus stocks for subsequent studies. Susceptibility to antiviral drugs was determined by plaque reduction assay with methods described previously. Drugs were obtained from the NIAID or purchased from Sigma Aldrich (St. Louis, MO). Cidofovir was a gift from Mick Hitchcock at Gilead Sciences (Foster City, CA).

Viral DNA was extracted from cells infected with Oka or 6/06/2 by standard methods. The TK gene was amplified using primers 5'-CAC CAT GTC AAC GGA TAA AAC CGA TGT AA-3' and 5'-GGA AGT GTT GTC ATG CCT AAC GGC ATT-3'; ORF62 was amplified using primers 5'-AAG TGG GTA AAC GCA GTC-3' and 5'-ATT ACT GTC GAC CCG AGA CC-3' and ORF28 was amplified using primers 5'-ATG TCA TCG TTT CAA TTT TGG-3' and 5'-ATT ACT GTC GAC CCG AGA CC-3'. Resulting PCR products were sequenced using a set of internal primers to sequence both strands of the DNA. The TK open reading frames from both strains were cloned into the pET151d bacterial expression vector (Invitrogen, Carlsbad CA) and the open reading frames were completely sequenced. The enzymes from both strains of VZV were expressed in *Escherichia coli* BL21 cells and were purified and assayed by methods described previously. Briefly, enzymatic activity was determined in a luciferase assay using thymidine as a substrate and used ATP consumption as a marker for enzymatic activity.

**RESULTS**

**Susceptibility to Antiviral Drugs.** The clinical isolate (6/06/2) was cultured, amplified, and its susceptibility to a set of representative antiviral drugs was determined by standard plaque assay using Oka as a fully susceptible control virus. The 6/06/2 isolate was fully susceptible to both foscarnet and cidofovir, whereas it was modestly resistant to ganciclovir (GCV) and highly resistant to both ACV and penciclovir (PCV) (Table 1). These results seem to be consistent with the clinical course including the inability of ACV to limit the infection.

**Molecular Characterization of 6/06/2.** Significant differences were observed in drug sensitivity between the clinical isolate and the Oka strain. Initial studies sought to confirm that the clinical specimen was derived from the vaccine strain. A previous report identified a polymorphism diagnostic of the vaccine strain, which is used by the vaccine manufacturer to confirm vaccine strain identity. This region of the clinical isolate was sequenced and did contain a *SalI* site characteristic of the Oka strain, indicating that the patient's isolate was derived from the vaccine virus. To characterize the mutations that the strain acquired to become resistant to the drugs, the coding regions of the TK and polymerase genes were completely sequenced. A single mutation was observed in 06/06/2, resulting in a T256A mutation in the amino acid sequence of the TK. Seven additional mutations were also identified in the coding sequence of the viral DNA polymerase. Amino acid mutations identified were Q532R in region IV, C566Y and 1593V in region C, and L193S and N887S in regions II and I, respectively. Two additional mutations, V121I and F182R, were also observed in the amino terminus of the polymerase. Mutations that confer resistance to ACV have been identified previously in the carboxyl terminus of the TK open reading frame near the T256A mutation in this isolate. To confirm that this mutation contributed to drug resistance, the open reading frames from Oka and from 06/06/2 were cloned into a bacterial expression vector, expressed in bacteria, and purified. The enzyme expressed from the Oka open reading frame was highly active with thymidine as a substrate and had a *V₅₀* of 6.8 μM min⁻¹ μg⁻¹, whereas the enzyme expressed from the 06/06/2 ORF did not exhibit detectable activity (<0.25 μM min⁻¹ μg⁻¹). Thus this mutation is likely sufficient to explain the observed resistance to ACV, PCV, and GCV that each require phosphorylation by this viral enzyme. The additional mutations in the polymerase gene are also similar to those reported in drug resistant strains, but were not investigated further. The TK mutation in this isolate is likely sufficient for the high level of antiviral resistance observed for this virus.

**DISCUSSION**

This case represents a second report describing chronic disseminated varicella disease caused by acyclovir-resistant Oka strain V2V, but it is the first to document recovery of a replication-competent Oka strain viral isolate from a patient. The only other published report of acyclovir-resistant Oka strain VZV also occurred in a 1-year-old child who began chemotherapy for neuroblastoma shortly after administration of the varicella vaccine. In both cases, the hyperkeratotic, verrucous appearance of the patient's rash is consistent with the well-described clinical entity of chronic, ACV-resistant wild-type VZV infection in immunocompromised patients. The clinical appearance of this rash should thus alert the clinician to the possibility of antiviral-resistant VZV, even if disease is believed to be caused by Oka strain varicella.

This case is the first report of a live viral isolate derived from Oka strain recovered and characterized from a patient with clinical suspicion for antiviral resistance, demonstrating in vivo viral replication during ACV therapy. In prior reports, DNA was isolated from clinical lesions and antiviral sensitivity was characterized based on the DNA sequences. Our inability to recover infectious virus from skin lesions after initiation of foscarnet therapy suggests that foscarnet was able to limit viral replication in vivo, although the patient failed to achieve complete resolution of symptoms until cessation of chemotherapy and reconstitution of the immune system.

The Oka strain varicella vaccine is highly attenuated and is generally accepted as safe in immune-competent individuals; however, its use is restricted in those with impaired cellular immunity or in close family contacts of such patients. Current recommendations state to withhold vaccination in patients with leukemia, lymphoma, or other malignancies in remission until at least 3 months after cessation of chemotherapy. These 2 cases of resistant varicella strain varicella in patients with malignancy argue the need for particular attention regarding status of varicella vaccination history at the time of cancer diagnosis in pediatric patients. Some experts recommend initiating prophylactic oral ACV (80 mg/kg/d) if an immunosuppressive event occurs within 4–6 weeks of vaccination and continuing ACV prophylaxis until completion of chemotherapy or the immunosuppressive event. Prompt treatment with
infrared ACV should be initiated at the first signs of infection to avoid development of resistance, even in Oka strain ZV. This virus likely became resistant in an environment of impaired immunity and inconsistent ACV dosing because of intolerance of oral therapy. The role of IVIG and varicella immunoglobulin (VarZig) in this setting is less clear.

Because of the grim prognosis of stage IV neuroblastoma, autologous transplantation is generally considered front-line therapy. The chance of wild-type varicella reactivation postautologous transplant may be as high as 48% in high-risk patients, although disseminated necrotizing disease is rare.14 The morbidity of CNS reactivation in the immunocompromised patient can be particularly severe, potentially leading to myelitis, encephalitis, and arteritis.15 These factors were considered heavily in the treatment of this patient for whom the risk of both varicella reactivation and neuroblastoma relapse were high. Our experience and that of Levin et al16 is that autologous transplantation may be feasible in similar cases after sufficient time has elapsed to permit immune reconstitution.

ACKNOWLEDGMENTS

The authors thank Dr. Myron Levin for helpful discussions during clinical management of this patient, and Dr. Kerry Parsons for her contribution to the review of this case. We would also like to thank the nurses, physicians, pharmacists, and other dedicated personnel involved in the care of this child.

REFERENCES


POLYOMA VIRUS HEMORRHAGIC CYSTITIS IN AN OTHERWISE NORMAL CHILD

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Abstract: We describe a case of polyoma virus hemorrhagic cystitis in a nonimmunosuppressed child. Polyoma virus infection was suspected because of abnormal urinary culture. Polyoma virus cystitis in nonimmunosuppressed children is self-limited, resolving spontaneously within 2 weeks.

Key Words: polyoma virus, hemorrhagic cystitis

Accepted for publication March 3, 2008.

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DOI: 10.1097/INF.0b013e318173d668

A cute hemorrhagic cystitis is a pediatric problem that has multiple etiologies.1 A bacterial etiology can be identified by urine culture. A virul etiology, however, requires special tests.2 We present a case of viral hemorrhagic cystitis where the diagnosis of polyoma virus was suspected because of abnormal cytology.

CASE PRESENTATION

A 3-year-old circumcised boy presented to his pediatrician with a 2-day history of pink urine, dysuria, increased frequency, and urgency. He had no prior medical problems. The physical examination was normal. Urinalysis revealed blood without casts; leukocyte esterase and nitrites were negative. Urine culture was sterile. An abdominal ultrasound examination was performed and showed a questionable area of bladder wall thickening; no urinary stones were identified. Urinary cytology showed a cluster of uroepithelial cells with smudgy chromatin, suggesting polyoma viral cytopathic effect. Confirmatory immunohistochemistry staining for the SV40 antigen was positive (Fig. 1). The patient was referred to the infectious disease clinic. On day 11 of symptoms, he complained only of dysuria. Urinalysis showed RBCs without casts. Urine cytology demonstrated polyoma cytopathic effect, which was confirmed by immunohistochemistry staining. Concurrent bacterial culture was negative. Urine viral culture for adenovirus and cytomegalovirus was negative. Over the next week, his dysuria resolved and follow-up studies (urinalysis, ultrasound, cytology, and immunohistochemistry) were negative. The patient has remained well during the last 5 years.

DISCUSSION

Causes of hemorrhagic cystitis include bacteria (especially Escherichia coli), viruses (ie, adenovirus, polyoma [BK] virus, influenza A, herpes simplex), chemical toxins, bleeding disorders,