Acyclovir-resistant varicella zoster virus (VZV) causes significant morbidity and mortality in immunosuppressed children. After pediatric hematopoietic stem cell transplant (HSCT), VZV disease is common and often atypical. Acyclovir (ACV)-resistant VZV is increasingly reported in immunocompromised populations, posing therapeutic dilemmas. Foscarnet is a therapeutic option in many of these cases—although few publications include pediatric patients. We report a pediatric HSCT patient with severe ACV-resistant zoster caused by an ACV-resistant VZV with a novel thymidine kinase (TK) mutation and the successful use of foscarnet salvage therapy.

CASE PRESENTATION

A 10-year-old girl with Fanconi anemia, presented 1 year after a second, matched unrelated donor HSCT with painful vesicular skin lesions on her left lower back, clustered in a dermatomal band. She was febrile and exhibited no signs of systemic infection. There was a history of VZV infection in early childhood supported by a VZV seropositive status pre transplant. She had received several months of ACV prophylaxis after each HSCT because of poor granulocyte count. A clinical diagnosis of herpes zoster was made, oral ACV (80 mg/kg/d) was initiated, and several lesions were cultured yielding VZV. Unfortunately, despite 2 weeks oral ACV therapy, new vesicles continued to develop over her trunk and lower extremities in a multidermal pattern, prompting hospital admission and high-dose (45 mg/kg/d) intravenous ACV.

After 1 week of intravenous ACV, new lesions continued to develop (culture positive for VZV) and ACV resistance was suspected. Therapy was switched to intravenous Foscarnet (120 mg/kg/d) while the VZV isolates were referred to the National Microbiology Laboratory (Winnipeg) for ACV resistance testing.

Response to intravenous foscarnet was dramatic. New lesions ceased to appear, and, within 5 days, all previous lesions were healing. Foscarnet therapy was continued for 4 weeks and was well tolerated. However, 1 week after stopping foscarnet, new lesions appeared and foscarnet was restarted. Again, the response to therapy was rapid and an additional 4-week course was planned. Unfortunately, the patient developed bacterial sepsis related to a central venous access device and died.

RESISTANCE TESTING

Genotypic ACV resistance testing was done by polymerase chain reaction amplification of the VZV TK (open reading frame, ORF 36) and DNA polymerase (ORF 28) genes using the following primer sequences: ORF 36 Forward = 5′-GACGTTGCGAACGTTG-3′; ORF 36 Reverse = 65950-65991 (5′-GGCGGATTAAAG-GATGTTG-3′); ORF 28 Forward = 46853-46884 (5′-TTTATTTTCCGGGAAAATCTG-3′); ORF 28 Reverse = 50763-50742 (5′-TAAACGGGATTACATATCGGC-3′). Amplcons of 1258 bp (ORF 36) and 3910 bp (ORF 28) were then sequenced. Sequences were compared with wild-type and ACV-resistant VZV strains. A novel 2 base-pair (TA at nt 375-376) deletion in the TK gene was detected—resulting in a frameshift and truncated TK protein. The mutated protein diverged from the wild-type TK sequence after AA-125 and was truncated from 361 amino acids (normal protein) to 162. No mutations were detected in the DNA polymerase gene.

In addition to the genotyping data, a phenotypic assay was developed to assess the mutant VZV strain for ACV susceptibility/resistance. The Merck vaccine strain (Varivax) was used as the wild-type strain with respect to ACV susceptibility. A 50 TCID50 inoculum of the clinical VZV isolate and the vaccine strain were added to MRC-5 cells (ATCC CRL-2222) in 24-well tissue culture plates, and adsorbed for 1 hour at 35°C. Minimal essential media containing 3-fold serial dilutions of ACV (acycloguanosine, A4669, Sigma, Oakville, Ontario) was added to the wells and inoculated for 5 days at 35°C. ACV concentrations were 0.1, 1, 3, 9, 27, 81, and 243 μM. Total DNA was harvested from each well using Qiamp extraction (Qiagen, Mississauga, Ontario). VZV was quantified by real-time polymerase chain reaction and standardized by comparison to β-globin as described previously. Viral strains were tested at each ACV concentration in quadruplicate. Two-way variance analysis indicated a statistically significant difference between the wild type and clinical isolates at 1, 3, 9, and 27 μM ACV concentrations (Fig. 1)—confirming the genotyping data’s evidence of ACV-resistance.

DISCUSSION

ACV-resistant VZV, is rare in immunocompetent individuals but has been described in immunocompromised patients. Forty-four clinical isolates of ACV-resistant VZV are described in the literature; largely in patients with acquired immunodeficiency syndrome (AIDS) (Table 1 available online). Clinically, ACV-resistant VZV infection has been defined by the persistence of lesions despite 10 days of therapy. Saint-Leger et al suggest that persistent lesions at day 10 may simply indicate the need for prolonged ACV in immunocompromised patients and that treatment failures after 21 days of therapy are more predictive of ACV resistance in this setting.

Laboratory detection of ACV resistance is a specialized process. Among phenotypic assays, plaque reduction remains the gold standard. Unfortunately, this assay can take weeks to perform and is influenced by cell lines, viral inoculum size, antiviral concentration, and user interpretation, making interassay comparison difficult. Phenotypic assays also have a limited ability to discriminate small proportions of ACV-resistant viruses within a larger heterogeneous population. In addition, passage of resistant virus in antiviral free cells during initial isolation removes selective pressure and may allow wild-type subpopulations to reemerge as the dominant population, masking resistant strains.
In genotypic assays, amplification and sequencing of a target gene allows comparison with wild-type virus to detect changes responsible for resistant phenotypes. Genotyping is rapid, more sensitive than phenotype assays, and allows detection of resistant viruses present in low numbers within a mixed population. Resistance mutations are well characterized for viruses such as human immunodeficiency virus and cytomegalovirus, but less data is available for herpes simplex virus and VZV.\(^\text{1,2,21}\)

The initial step in ACV phosphorylation to its active triphosphorylated form is catalyzed by viral TK.\(^\text{2,13,32,33}\) TK gene mutations leading to a nonfunctional, truncated or absent protein (TK\(^-\)), or mutations resulting in decreased production of TK (TK\(^\text{K}\)), account for 95% of ACV-resistant mutants.\(^\text{5,24}\) Rare DNA polymerase mutations account for others. TK gene mutations occur most frequently at "mutagenic hot spots" in the adenosine triphosphate and nucleoside-binding sites.\(^\text{24}\) The 2 base-pair (T-A at nt375-6) deletion in the TK gene detected in our case is, to our knowledge, novel. VZV isolates have been reported, however, with TK gene additions or deletions leading to similarly truncated proteins and the TK\(^-\) phenotype seen in this case.\(^\text{1,12,22,26,31}\)

Our patient responded rapidly to foscamet—the drug of choice for ACV-resistant VZV strains.\(^\text{20,32}\) A pyrophosphate analogue, foscamet directly inhibits viral DNA polymerase without requiring phosphorylation to be active.\(^\text{10}\) Renal toxicity and availability only as an intravenous drug limits its prophylactic role in patients with recurrences of ACV-resistant VZV.\(^\text{12}\)

Recently, emergence of foscamet-resistant strains have been reported.\(^\text{24}\) Cidofovir, an acyclic nucleoside phosphonate that does not require TK for conversion to the active form, is often effective against both TK-deficient and foscamet-resistant mutants.\(^\text{31,37}\)

CONCLUSIONS

Our case illustrates the need for clinicians to have an index of suspicion for ACV-resistant VZV in immunocompromised patients with VZV infections—particularly those with prior ACV exposure. Laboratory testing for ACV resistance is a specialized procedure but is valuable both for augmenting clinical diagnoses and for understanding the molecular epidemiology of the problem. The phenotypic characterization of the novel mutation we described (TA at nt375-6) supports previous reports that a single mutation in the TK gene can confer ACV resistance. Foscamet can be a life-saving alternative for patients with ACV-resistant VZV infections.

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REFERENCES


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LIPOSOMAL AMPHOTERICIN B ASSOCIATED WITH SEVERE HYPERPHOSPHATEMIA

Scott M. Sutherland, MD, * David K. Hong, MD, † Jay Balogas, MD, † Kathleen Gutierrez, MD, ‡ Christopher C. Dvorak, MD, † and Minnie Sarwal, MD, PhD*  

Abstract: We report 4 patients who developed hyperphosphatemia while receiving liposomal amphotericin B to treat an invasive fungal infection. Resolution of the hyperphosphatemia occurred after transition to amphotericin B lipid complex. This phenomenon may occur more commonly in patients with mild to moderate renal insufficiency.

Key Words: antifungal, invasive fungal infection, amphotericin B, hyperphosphatemia

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From the Divisions of *Pediatric Nephrology, †Pediatric Infectious Diseases, and ‡Pediatric Stem Cell Transplantation, Department of Pediatrics, Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA. Address for correspondence: Scott M. Sutherland, Division of Pediatric Nephrology, Department of Pediatrics, 300 Pasteur Drive, Stanford, CA 94305-5208. E-mail: sutherm@stanford.edu.

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The standard treatment of invasive fungal infections in children is the polyeone amphotericin B deoxycholate or one of its lipid formulations, as they have a broad-spectrum of activity against Candida spp., Aspergillus spp., Fusarium spp., and fungi in the Zygomycetes class. These agents can cause hypokalemia and hypomagnesemia secondary to renal tubule injury. Hyperphosphatemia may be an under-recognized problem with administration of liposomal amphotericin B deoxycholate (Ambisome, Astellas Pharma USA, Inc., Deerfield, IL). We report 4 children who developed hyperphosphatemia while receiving liposomal amphotericin B.

CASE 1

An 11-year-old boy with high-risk pre-B-cell acute lymphoblastic leukemia (ALL) in remission was admitted with fever and neutropenia. Although his induction chemotherapy had been completed, he continued to have fever and hypotension. A chest radiograph revealed a right lower lobe infiltrate, and blood cultures grew Aspergillus fumigatus. He was started on high-dose liposomal amphotericin B for invasive aspergillosis. Several days after starting treatment, his serum phosphorus level increased to 12.0 mg/dL from his baseline level of 5.5 mg/dL. He was hypokalemic and hypomagnesemic. During therapy with liposomal amphotericin B, the patient's serum creatinine increased from 1.0 mg/dL to 1.6 mg/dL. The patient showed clinical improvement with resolution of fever and leukocytosis. After 7 days of liposomal amphotericin B therapy, the patient's serum phosphorus level decreased to 6.9 mg/dL. His serum creatinine level decreased to 1.2 mg/dL, and his potassium and magnesium levels normalized. The patient was subsequently transitioned to amphotericin B lipid complex. The hyperphosphatemia resolved on this agent.

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