Clinical and Virologic Characterization of Acyclovir-Resistant Varicella-Zoster Viruses Isolated from 11 Patients with Acquired Immunodeficiency Syndrome

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We studied the clinical resistance to acyclovir of infections with varicella-zoster viruses (VZV) in patients with acquired immunodeficiency syndrome, and we correlated it to virologic analyses. Eleven patients with VZV infections (treated with acyclovir, 30 mg/kg/day, given intravenously, or 4 g/day, given orally) were included in the study because of the failure of 10 days of acyclovir therapy. Susceptibility of VZV isolates to acyclovir was tested using a plaque reduction assay to determine the 50% inhibitory concentration (IC50) of acyclovir and the SL50 (IC50 of the patient isolate/IC50 of the reference strain) to acyclovir. The thymidine kinase (TK) gene, which supports the resistance, was sequenced on amplified products. Only 3 patients had a significant increase in the IC50, as compared with the IC50 of the reference strain (SL50 of ≥4), and a mutation in the TK gene. For the other 8 patients, the clinical resistance was not confirmed by the virologic results: the SL50 was <4, and no mutation was detected in the TK gene. Because no acyclovir-resistant strain appeared during a shorter period of time, we suggest an increase in the duration of the treatment to 21 days before acyclovir resistance is suspected.

The varicella-zoster virus (VZV) is responsible for herpes zoster in immunocompromised patients with HIV. According to published guidelines, VZV infections are treated with a 10-day course of acyclovir, administered intravenously, with at least 10 mg/kg t.i.d. or 1500 mg/m² per day [1, 2], or orally, at 800 mg 5 times per day [1, 3, 4]. Acyclovir-resistant VZV infections are defined by the occurrence of persistent lesions after 10 days of acyclovir therapy [5, 6]. Since the first case was reported by Pawha et al. [7] in 1988, only 35 cases of infections with acyclovir-resistant strains have been described in the literature. The main risk in profoundly immunocompromised patients is visceral dissemination, especially in a neurological location. Acyclovir-resistant VZV infections have a poor prognosis, with nearly all patients dying within 6 months of diagnosis [5, 7–11]. The genetic support of acyclovir resistance is often a single mutation in the viral thymidine kinase (TK) gene, which is required to phosphorylate acyclovir to acyclovir monophosphate. The second and third phosphorylations leading to the active form of acyclovir are performed by cellular thymidine kinase. Point muta-
Table 1. Main features of the 11 patients with clinical acyclovir-resistant varicella-zoster virus (VZV) infections.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Main disease (ICD4 cell count, ×10^6 cells/L)</th>
<th>Clinical aspect of VZV infection</th>
<th>Prior anti-VZV treatment</th>
<th>Dosage (duration)</th>
<th>Result</th>
<th>Results of alternative therapy</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV^a (9)</td>
<td>1 isolated keratotic lesion persistent after zoster</td>
<td>Oral Acy for zoster; no maintenance therapy</td>
<td>iv Acy, 30 mg/kg/day (21 days), followed by oral Acy, 4g/day (7 days)</td>
<td>No efficacy</td>
<td>Healing with Pfa</td>
<td>Patient died 6 months later without zoster recurrence</td>
</tr>
<tr>
<td>2</td>
<td>HIV^a (290)</td>
<td>3 chronic lesions, large and ulcerated</td>
<td>Acy, oral or iv, or Pfa for several cases of varicella and zoster during the last year of treatment</td>
<td>Acy and Pfa, 3 times per week, given simultaneously</td>
<td>Improvement without healing</td>
<td>Pfa for 45 days; healing</td>
<td>Patient died 4 months later without zoster recurrence</td>
</tr>
<tr>
<td>3</td>
<td>HIV (7)</td>
<td>1 chronic lesion (diameter, 2–3 cm) persistent after healing of a disseminated zoster</td>
<td>Oral and iv Acy for disseminated cutaneous zoster and cutaneous herpes; curative and maintenance therapy</td>
<td>iv Acy, 30 mg/kg/day (several months)</td>
<td>Persistence of the skin lesion and dissemination to lung and pancreas.</td>
<td>Improvement began with Pfa</td>
<td>Patient died 1 month later</td>
</tr>
<tr>
<td>4</td>
<td>HIV (10)</td>
<td>1 persistent isolated crusted lesion after healing of a zoster</td>
<td>Oral Acy for several months for chronic anal herpetic lesion; curative and maintenance therapy</td>
<td>iv Acy, 30 mg/kg/day (14 days)</td>
<td>Improvement starting on day 14</td>
<td>No</td>
<td>Slow improvement with oral Acy, 800 mg/day, in 2 months of maintenance therapy</td>
</tr>
<tr>
<td>5</td>
<td>HIV (1)</td>
<td>Usual zoster</td>
<td>Oral Acy for genital herpes lesion; iv Acy for zoster</td>
<td>iv Acy, 30 mg/kg/day (1 month)</td>
<td>Improvement without healing</td>
<td>No</td>
<td>No data available about zoster evolution after the first month of treatment</td>
</tr>
<tr>
<td>6</td>
<td>HIV (10)</td>
<td>Chronic varicella with only a few hyperkeratotic lesions</td>
<td>800 mg of oral Acy for herpes lesion for 1 month</td>
<td>iv Acy, 30 mg/kg/day and 45 mg/kg/day (21 days)</td>
<td>No efficacy</td>
<td>Incomplete improvement with Pfa; relapse with maintenance therapy</td>
<td>Efficacy with 1 month of oral Sor, 40 mg/day; healing in 10 days; 3 months without relapse</td>
</tr>
<tr>
<td>Case</td>
<td>HIV Type</td>
<td>Diagnosis</td>
<td>Treatment</td>
<td>Acyclovir Dose</td>
<td>Outcome</td>
<td>Notes</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>HIV (130)</td>
<td>1 crusted isolated lesion</td>
<td>Oral and iv Acy in curative therapy for zoster; 2 g/day in maintenance therapy</td>
<td>iv Acy, 30 mg/kg/day (18 days)</td>
<td>Improvement without healing</td>
<td>No Maintenance therapy with Acy, 200 mg 5 times per day; persistence of improvement, but the patient died 3 weeks later</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>HIV (10)</td>
<td>Usual zoster</td>
<td>1 previous zoster 2 months earlier; no data about curative treatment; no maintenance therapy</td>
<td>Oral Acy, 4 g/day (10 days)</td>
<td>Improvement without healing</td>
<td>No Healing with Acy, 1 g/day, in 20 days</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>HIV (10)</td>
<td>Usual zoster associated with herpes lesion</td>
<td>Oral Acy and Vcv and iv Acy for previous zoster; Pfa for treatment of a persistent zoster after 10 days of oral Acy, 4 g/day</td>
<td>iv Acy, 30 mg/kg/day (10 days)</td>
<td>No efficacy</td>
<td>Healing, but relapse, with Pfa</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>HIV (3)</td>
<td>Fourth relapse of varicella within the previous 5 months; 2–3 lesions persistent on the face</td>
<td>iv Acy for the 3 previous relapses; Vcv in maintenance therapy between the third and fourth relapse</td>
<td>iv Acy, 750 mg/day (17 days); Vcv in maintenance therapy</td>
<td>Healing</td>
<td>No No relapse; HAART was introduced, and CD4+ count, lymphocyte count, and virus load improved simultaneously</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>HIV (28)</td>
<td>Multidermatomal zoster with hyperkeratotic aspect</td>
<td>Oral and iv Acy alternatively with Pfa for a recurrent zoster; patient was simultaneously treated with Pfa and Acy when clinical resistance to Acy occurred</td>
<td>iv Acy, 1500 mg/m² per day (14 days)</td>
<td>No improvement</td>
<td>Failure with Pfa</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Acy, acyclovir; HAART, highly active antiretroviral therapy; Pfa, foscarnet; Sor, sorivudine; Vcv, valaciclovir.

a. Patient also had sinus lymphoma and received corticotherapy and chemotherapy.

b. Patient was a 6-year-old child.

c. Patient also had lymphoma and received chemotherapy.

d. Patient also had orbit lymphoma and received corticotherapy and chemotherapy.

e. Patient was a 4-year-old child.

f. Concentration of Pfa that reduced viral replication by 50%, 350 mM.
tions are located anywhere within the TK gene. Inside the pu-
tative functional sites (an adenosine triphosphate–binding site
and nucleotide-binding site), mutations consist of a single
amino acid substitution; however, outside of these sites, mu-
tations consist more often of a premature “codon stop” oc-
currence [12, 13].

In vitro acyclovir resistance is usually correlated with clinical
resistance, except in a few cases [8, 12]. We conducted a re-
verspective study of 11 cases in which clinical VZV infections
were clinically resistant to acyclovir, and we analyzed the cor-
relation between clinical data and the virologic results.

MATERIALS AND METHODS

Patients. Inclusion criteria consisted of clinical acyclovir re-
sistance, which was defined by the appearance of persistent
lesions despite receipt of acyclovir therapy (>30 mg/kg/day; 1
patient received 4 g/day) for at least 10 days, on the basis of
the previously reported definition of acyclovir resistance [5, 6].
The clinical information was collected by means of medical
observations provided from several medical centers (Pitié-Sal-
petrière Hospital, Paris; Bordeaux Hospital, Bordeaux; Ambro-
ise Paré Hospital, Boulogne; and Necker-Enfants Malades Hos-
pital, Paris). Eleven patients were selected. The main clinical
features are summarized in table 1. Exclusion criteria consisted
of receipt of inappropriate therapy (less than 10 days or 30 mg/
kg of acyclovir per day) and the lack of VZV isolation in the
cutaneous lesions.

VZV isolates. VZV samples were obtained and submitted
to the Virology Department at La Pitié-Salpêtrière Hospital
to have an in vitro acyclovir susceptibility assay performed when
acyclovir therapy had failed. For each patient, only 1 isolate,
which had been obtained when acyclovir resistance occurred,
was available. VZV isolates were obtained after inoculation on
fibroblastic cells (MRC-5). The presence of VZV infection was
recognized by its typical cytopathic effect on MRC-5 cells. Two
susceptible strains were used as references: either Oka (vaccine
strain) or our laboratory reference strain, Cha, which was ob-
tained from a child with chickenpox who had never received
acyclovir.

Antiviral susceptibility assay. Susceptibility of VZV to
acyclovir was evaluated by the determination of the concen-
tration of acyclovir that reduces viral replication by 50% (IC50)
using the late antigen reduction assay [14]. In brief, this assay
was performed with different dilutions of the cell-associated
virus cultivated with different antiviral concentrations; 48 h
after the infection, a reduction in the synthesis of VZV late
antigen was detected by immunoperoxidase staining with spe-
cific antibodies. Foci were counted under an optical micro-
scope. IC50 was determined as described elsewhere [14]. The
test was performed—generally in duplicate, but for 2 of the 11
patients (patients 5 and 7), it was performed only once—and
compared with a susceptible reference strain that was analyzed
in the same experiment. According to data published elsewhere
[7, 8, 10, 12–15], we selected a susceptibility index (SI50; IC50
patient isolate/IC50 reference isolate) of >4 as the threshold to
to screen resistant and susceptible VZV isolates. SI50 was calculated
in the same experiment. According to data published elsewhere
[14, 17]. The DNA sequencing was performed on both strands
by a company that uses the dye terminator assay and the ABI
sequencer (PE Applied Biosystems), except in patient 1, who
has been described elsewhere [14]. The DNA sequence was
analyzed using the software sequence navigator, and each pa-
tient’s TK gene was compared with the TK gene of the Dumas
strain, the first acyclovir-susceptible strain described in the lit-
terature [18].

RESULTS

Ten of 11 patients selected had previously received treatment
with acyclovir for at least 10 days (table 1). For patient 8, no
data were available regarding the treatment of his initial zoster,
but it was typical to treat all cases of VZV infection in patients
with HIV. This prior treatment had been administrated either
punctually or in the long term for herpes recurrence or for
anterior herpes zoster before suspicion of VZV resistance to
acyclovir. Among the 11 patients, 4 had herpes zoster eruption
with the usual aspects, and 7 had hyperkeratotic, crusted, and
ulcerated lesions, which are often limited to 3 or 4 lesions. The
mean lymphocyte CD4+ cell count was 45 × 10^6 cells/L (range,
1–290 × 10^6 cells/L), and the median lymphocyte CD4+ cell
count was 10 × 10^6 cells/L. The duration of the acyclovir treat-
ment ranged from 10 days to several months (median duration
of acyclovir therapy, 18 days). For at least 4 patients (data were
unknown for patient 5), acyclovir therapy was continued, either
intravenously or orally, and led to healing (complete for 3
patients and partial for patient 7) of the lesions. For the 6 other
patients, the alternative antiviral drugs of foscarnet or sor-
vudine were introduced (table 1).
Results of the phenotypic study are summarized in table 2. IC_{50} and SI_{50} values were between 10 μM and 155 μM and <1 and 13, respectively. Three of 11 patients had an SI_{50} of ≥4 (the IC_{50} values were >100 μM, 23 μM, and 155 μM for patients 1, 2, and 3, respectively).

A comparison of the TK gene sequences of all VZV isolates to that of the Dumas strain (the reference VZV strain for genotypic analyses) showed that each of the isolates had a substitution of S-288→L. This amino acid change has been found in nearly all VZV isolates described elsewhere by researchers other than Dumas [13, 15, 16, 19]. Only 3 patients (patients 1–3) with an SI_{50} of ≥4 had an additional mutation in the TK gene (table 2). The changes in the TK gene differed from those obtained from other patients: for each isolate, a unique mutation was found. Among these 3 VZV-resistant isolates, 2 substitutions (in patients 1 and 2) and 1 deletion (in patient 3) were detected in an area in which other mutation points have been described elsewhere.

**DISCUSSION**

Among the 11 patients with clinically suspected acyclovir-resistant VZV infection, only 3 (patients 1–3) had an acyclovir-resistant VZV infection, as defined by both phenotypic and genetic characterization. For these 3 patients, it was noted that the SI_{50} was ≥4. These results are in agreement with literature analyses that have shown that an SI_{50} threshold of ≥4 may discriminate a resistant VZV strain from a susceptible one. In the present study, both IC_{50} and SI_{50} were used to characterize in vitro susceptibility, because IC_{50} varies with the methods used and within the same method because of difficulties for standardization. The variation of IC_{50} could be as large as 20-fold between 2 different methods for the same isolate, as was reported by Snoeck et al. [10] and Standing-Cox et al. [20]. Subsequently, we think that it is not always possible to deduce the susceptibility or the resistance of a VZV strain from nothing more than the IC_{50} value. It was observed that the SI_{50} results for all acyclovir-resistant strains of VZV were correlated with the drug-susceptibility status, no matter what method was used [7, 8, 10, 12–15].

For patient 2, the very terminal mutation point (substitution L332→P) can explain why the IC_{50} and SI_{50} are weakly increased. This mutation is located in an area that has already been implicated in acyclovir resistance by Boivin et al. [12] (strain 17 L), who reported a premature codon stop appearance at the amino acid 333 position near our mutation (amino acid 332). According to what is known about the herpes simplex virus TK, this terminal site on the TK is important for the enzyme stability [21]. With regard to this 6-year-old patient, it is noteworthy that the CD4+ count was relatively high (290 cells/mm^3), which stands in contrast with data published elsewhere [5, 7, 9, 11] and with data regarding 2 of our other patients (table 1). However, because of maternofetal HIV transmission, we assume that this child had not established complete VZV immunity after his primary VZV infection.

The severity of immunosuppression is an important risk factor for the occurrence of resistance to acyclovir. Thus, patient 1, who had additional factors of immunodepression, such as lymphoma, chemotherapy, and corticotherapy, exhibited the shortest delay (3 weeks with iv acyclovir) for the occurrence of acyclovir resistance.

Patient 3 was the only patient with a disseminated infection that began with a uniquely persistent and crusted lesion after healing of herpes zoster. The resulting mutation consisted of a truncated protein TK in which only 183 amino acids persisted among the 341 of the complete protein. Because of the large size of the deletion, it can be hypothesized that this deletion conferred a marked reduction of susceptibility to acyclovir (table 2).

These 3 patients (patients 1–3) died within 6 months of presentation, as had other patients reported elsewhere. Patient 3 died with the persistence of lesions after 1 month of treatment with foscarnet. Patients 1 and 2 were cured with foscarnet; however, they also died, without VZV recurrence 6 and 4 months after the lesions healed, respectively. Foscarnet, a drug with a metabolism not dependent on TK, is the main alternative therapy when acyclovir fails, and it cured 3 (60%) of 5 patients in the study by Safrin et al. [5] and 10 (70%) of 13 patients in the study by Breton et al. [11].

**Table 2. Results of phenotypic and genotypic characterization of the 11 varicella-zoster virus (VZV) isolates recovered from patients infected with VZV that was clinically resistant to acyclovir.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>IC_{50}, μM</th>
<th>SI_{50}</th>
<th>Nucleotide</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;100</td>
<td>&gt;6, 2</td>
<td>C 907→T</td>
<td>Q 303→stop</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>4</td>
<td>T 995→C</td>
<td>L 332→P</td>
</tr>
<tr>
<td>3</td>
<td>155</td>
<td>13</td>
<td>Del 535–539</td>
<td>Stop 183</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>8b</td>
<td>1, 4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>0, 8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>10b</td>
<td>0, 8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>&lt;1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>3, 2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>3, 5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>31</td>
<td>1, 8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE. Del, deletion; IC_{50}, concentration of acyclovir that reduced viral replication by 50%; ND, no data; SI_{50}, IC_{50} of the patient isolate/IC_{50} of the reference isolate; —, no change, in comparison with the published sequence of Dumas VZV strain [18] and excluding the widespread S288→L mutation.

a Experiments were performed in monoplicate.
We were surprised that, for the 7 other patients (patients 4–10) who were also selected because of persistent lesions after 10 days of acyclovir therapy, clinical resistance was not confirmed by virologic results. It is well known that clinical aspects of lesion caused by acyclovir-resistant VZV are often atypical—they are few, crusted, hyperkeratotic, and localized [1]. However, these aspects are not specific to antiviral resistance. Patients 4–10 had lesions that were undistinguishable from those observed in patients with virologic resistance (patients 1–3). Virologic resistance cannot be deducted on the basis of any clinical feature of the lesions. The chronic aspect seems to be supported by a particular pattern of VZV gE and gB gene expression [22]. Indeed, in our study, patients 4, 7, 8, and 10 had healing with the prolongation of acyclovir therapy, which is in accordance with our virologic results of acyclovir susceptibility. This clinical course suggests that the duration of the treatment needs to be increased to >10 days before the presence of an acyclovir-resistant VZV strain is suspected. In a study published elsewhere, patient 6 had been cured by use of sorivudine, a drug with a metabolism dependent on viral TK with an increased activity 2000–5000-fold greater than that of acyclovir [23].

Patient 9 had an unusual profile of susceptibility. Although an SI₅₀ was <4, the IC₅₀ is high for a susceptible isolate; we hypothesize that this isolate is, in fact, a mixed population composed of both acyclovir-resistant and acyclovir-susceptible strains. The acyclovir-resistant strain is proportionally sufficient to increase the IC₅₀, but it is in a proportion too weak to be sequenced on amplified products. The technique used allows detection of a double population only if the minor population is >20%.

If one takes into account patients 4–11, who had virologic acyclovir susceptibility, and patients 4, 7, 8, and 10 in particular, who simply required a longer time to heal with acyclovir, because the persistence of VZV infection was mainly the result of host deficiency, we suggest the introduction of the notion of “delay of healing.” So, on one hand, it is necessary to continue acyclovir, a weakly toxic drug, with optimal dosage and for a longer period to allow the healing of such lesions, instead of switching to foscarnet, a highly toxic drug. On the other hand, treatment has to be switched early enough and prior to occurrence of an in vitro acyclovir-resistant VZV strain. No resistant strain was found in our study or in the literature in a delay shorter than 3 weeks. Subsequently, we suggest that the precedent definition of VZV acyclovir resistance, suspected after 10 days, should be modified, and that the duration of acyclovir treatment should be extended to 3 weeks before resistance is suspected. In vitro susceptibility should be determined at the same time to screen resistant isolates from sensitive ones.

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References


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