Viremia in Acute Herpes Zoster

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Background. A phase 2 trial was conducted to evaluate the efficacy of a topical antiviral, sorivudine, as an adjuvant to valacyclovir for the treatment of acute herpes zoster.

Methods. In this randomized, placebo-controlled, double-blind trial, 25 patients were treated with either sorivudine or placebo cream. All patients began 7 days of valacyclovir treatment on day 3. Zoster lesion swab samples and samples of peripheral blood mononuclear cells were collected periodically throughout the study and were analyzed for varicella-zoster virus (VZV) DNA by use of both qualitative and real-time polymerase chain reaction. Serum samples collected periodically throughout the study were analyzed for VZV DNA by use of real-time polymerase chain reaction.

Results. VZV DNA was detected in all 3 sample types, and the number of viral copies correlated with the progression of herpes zoster. No statistically significant differences were seen between the placebo- and sorivudine-treated groups with respect to clinical characteristics or laboratory test results.

Conclusion. The detection of VZV DNA in the serum and peripheral blood mononuclear cells of all 25 zoster patients documents that viremia is a common manifestation of herpes zoster. Sorivudine cream appears to be a safe and well-tolerated adjuvant therapy; however, further phase 2 studies are needed to determine its clinical efficacy for the treatment of herpes zoster.

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Varicella-zoster virus (VZV) infection is exceedingly common; ~95% of the population becomes infected at some point in their lives. Primary infection with VZV manifests as the rash of varicella (chickenpox), generally at a young age, followed by establishment of latency in a dorsal root ganglion [1, 2]. Reactivation of latent VZV produces the clinical syndrome of herpes zoster (HZ), or shingles, which has a lifetime incidence of 10%–20% [3].

HZ manifests as a unilateral eruption along 1 or 2 contiguous dermatomes that is generally preceded by prodromal pain and parasthesia. The eruption lasts ~7–10 days and progresses from erythematous macules and papules to vesicles, then to pustules, and finally crusts over. However, HZ-associated pain can continue for months or even years after the acute clinical presentation. Approximately 5% of patients also suffer from systemic symptoms such as fever, fatigue, and malaise [4]. The incidence of HZ increases steadily with age and causes significant morbidity, especially in patients with decreased cell-mediated immunity, such as elderly individuals and immunosuppressed patients [1]. Studies have found an overall incidence of 1.2–3.6 cases per 1000 people per year [5–8], with the incidence increasing to 5 cases per 1000 in subjects more than 75 years of age [9].

The systemic symptoms involved in acute HZ suggest viremia, but VZV has rarely been consistently detected in the blood of otherwise healthy patients with shingles [10]. Documentation and quantification of viremia in patients with acute HZ can assist in our understanding of the disease and may provide a tool for assessing the efficacy of new antiviral drugs. Prodromal pain often precedes the classic erythematous and vesicular eruption of HZ by days or weeks [1], and even when clinicians strongly suspect HZ, they must wait to initiate
treatment until the vesicles present and confirm the diagnosis. The vesicular eruption is actually a late sign in the course of infection and having a method for early detection would be invaluable. Because HZ is a painful and often debilitating disease with the possibility of severe complications, especially in immunocompromised patients, the ability to diagnose HZ early and initiate treatment quickly could help prevent some of these sequelae. Mehta et al. [11] have demonstrated the clinical value of early detection of impending HZ. Detection of viremia may also allow for documentation of zoster sine herpete, the syndrome of pain and paresthesia along a dermatome due to HZ but not accompanied by the development of cutaneous findings.

Initiation of treatment within the first 72 h of vesicle formation is important for maximizing antiviral efficacy, reducing the time required for lesions to heal, and minimizing HZ-associated pain. Current treatment consists of oral therapy with an antiviral, such as acyclovir, valacyclovir, or famciclovir, taken for a 7-day period. The first antiviral agent approved to treat HZ, acyclovir (administered in a dose of 800 mg 5 times daily for 7–10 days), was introduced in 1983 [12]. Unfortunately, patient compliance tends to decrease as the number of daily oral doses increases; this fact, along with poor bioavailability and the inconvenience of taking the drug 5 times a day, led to the development of valacyclovir (administered in a dose of 1 g 3 times daily for 7 days) and famciclovir (administered in a dose of 500 mg 3 times daily for 7 days) [12]. These systemic medications are currently the therapeutic standard of care. Clinicians do not agree about whether adjuvant topical therapy is necessary or what topical therapy to use; patients may be advised to use an array of nonprescription and nonmedicated products, such as moisturizers, cooling lotion preparations containing menthol, and/or oatmeal-based soaks. No topical antiviral has yet been approved for treatment of shingles. This study evaluated the efficacy of adjuvant therapy with the topical antiviral sorivudine.

**SUBJECTS, MATERIALS, AND METHODS**

**Study Design**

The study was a phase 2, double-blind, randomized, placebo-controlled trial sponsored by aRigen Pharmaceuticals (Japan). The study compared ARYS-01 (sorivudine) 3% cream to placebo cream, both applied twice daily to the affected dermatome for 7 consecutive days. Twenty-five subjects ≥18 years of age who were diagnosed within the first 96 h after HZ rash onset were included. A central institutional review board approved the protocol, and the human experimentation guidelines of the United States Department of Health and Human Services were followed. Written informed consent was obtained from each subject. VZV infection was confirmed by use of polymerase chain reaction (PCR) of lesion samples obtained prior to beginning treatment. Exclusion criteria included HZ ophthalmicus, pregnancy, breastfeeding, use of cytotoxic drug therapy within the previous 3 months, use of 5-fluorouracil within the previous 6 months, use of topical or oral antiviral therapy within the previous 2 weeks, a history of corticosteroid-induced immunodeficiency, a history of malignancy, clinically significant renal insufficiency, liver enzyme levels ≥3 times the upper limit of normal, a history of intolerance or hypersensitivity to the cream components, current significant skin disease within the affected dermatome, seropositivity for human immunodeficiency virus, hepatitis B or C, a history of alcohol or substance abuse, or recent participation in any other investigational drug trial. Women were required to have a negative pregnancy test result at the time of screening to be enrolled, and they were required to use a highly effective contraceptive method during the course of the trial.

Subjects were screened and enrolled, and they began treatment with either ARYS-01 cream or placebo cream on day 1. During the 7 days of treatment with the cream, the patients were seen on days 1, 3, and 5. On the evening of day 3, all subjects began 7 days of oral valacyclovir therapy (1 g 3 times daily). Patients were also seen on day 8 (the day after the last application of the cream). Three follow-up visits, after the end of both topical and oral antiviral therapy, were conducted on days 14, 18, and 21. At each visit, the following assessments were conducted: clinical HZ rash assessment, local cutaneous tolerability assessment, and HZ-associated pain assessment using the Brief Pain Inventory tool [13]. A time line for the study is shown in figure 1.

HZ lesion swab samples were collected prior to treatment on day 1 and on days 3, 5, 8, and 14; swab samples were analyzed for the presence of VZV DNA by use of both qualitative PCR and real-time PCR. Blood samples for the measurement of serum VZV DNA levels were obtained on day 1 before the first application of topical cream and on days 3, 5, 8, and 14. Peripheral blood mononuclear cell (PBMC) samples obtained on days 1, 3, 5, 8, and 28 were analyzed by use of qualitative VZV PCR and samples obtained on days 1, 3, 5, and 8 were analyzed by use of real-time PCR.

**Laboratory Methods**

**DNA extraction.** DNA was extracted from the swab and PBMC samples using the Puregene DNA Purification Kit (Gen- trax Systems). Viral DNA was extracted from serum samples using ViralXpress Nucleic Acid Extraction Reagent (Chemicon International). The DNA concentration in swab samples was determined using the Quant-iT High-Sensitivity DNA Assay Kit (Molecular Devices–Invitrogen). The DNA concentration in PBMC samples was determined using QUANT-iT DNA Broad-Range Assay Kit (Molecular Devices–Invitrogen). The
Fluorescence was measured using the SPECTRAmax GEMINI XS Microplate Spectrofluorometer (Molecular Devices).

**Qualitative PCR.** The quality of amplifiable DNA was determined by PCR of the β-globin reference gene; this process has been described elsewhere [14, 15]. The samples, along with positive, negative, and reagent controls, were used for nested VZV PCR with primer sets derived from VZV-ORF29, as described elsewhere [16]. The PCR products were analyzed by use of gel electrophoresis on 2% SeaKem-LE agarose, and the PCR products were visualized with ultraviolet light after ethidium bromide staining.

**Real-time PCR.** Real-time PCR was used to quantify VZV DNA with the TaqMan 7900 DNA sequence detection system (Applied Biosystems). PCR was performed with the TaqMan PCR kit (PE Biosystems) using fluorescence-based simultaneous amplification and production detection. The primer and probe were derived from gene 63 [11, 17, 18].

**Statistical analysis.** All subjects who received ≥1 doses of the assigned treatment were included in the analysis. Pairwise associations among viral counts for lesion, PBMC, and serum samples were conducted within each time point using the Somers’ D measure of association. The Somers’ D measure is a nonparametric method of assessing consistency between disparate quantitative measures. The interpretation of Somers’ D is similar to that of Pearson’s r correlation coefficient, with D values ranging from negative 1.0 to positive 1.0; 0 indicates no association, and D values close to negative 1 or positive 1 indicate negative or positive association, respectively.

To assess whether there were changes in the viral load of PBMC and serum samples over time or by treatment group, we submitted the repeated observations to separate 0-inflated negative binomial regression analyses, with robust standard error estimates accounting for repeated observations within each single subject. The lesion data were highly skewed, as is typical for such data, and we have used a log transformation to eliminate some of the statistical skew. However, these transformed data retained their extreme variability, rendering parametric regression techniques inappropriate. We thus used median (i.e., least absolute value) regression to compare median changes in the data over time. Our models for all 3 outcomes included main effects for day and group as well as an interaction term so that we could determine whether there were time effects, group differences, or interaction effects.

**RESULTS**

The demographic characteristics of the 25 patients enrolled in the study are listed in table 1. The age of the subjects ranged from 21 to 79 years; 15 (60%) were female, and 10 (40%) were male. Of these 25 patients, 12 received sorivudine and 13 received a matching placebo cream; all patients received oral valacyclovir therapy. All subjects were diagnosed with acute HZ, were enrolled within 96 h after onset, and had PCR results positive for VZV DNA during the course of the study. A wide range of dermatomal distributions was found among the patients. Analysis of VZV copy numbers in the lesion, PBMC, and serum samples revealed significant decreases over time (\(P<.01, <.05, \) and \(.01\), respectively) (figure 2). However, there was no statistically significant difference between the placebo and the treatment groups with respect to VZV copy numbers.

There were also no statistically significant differences between the treatment and placebo groups with respect to laboratory test results, treatment tolerability, or incidence of resolution of the lesions. The mean number of VZV copies per milliliter

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**Figure 1.** Study timeline indicating study design and times that samples were obtained from patients with herpes zoster. PBMC, peripheral blood mononuclear cells.
Table 1. Demographic characteristics of patients with herpes zoster and dermatomal distribution of lesions.

<table>
<thead>
<tr>
<th>Patient number</th>
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DNA solution in the lesion swab samples was lower in the sorivudine group than in the placebo group. During the first 5 days of the study, there was no worsening of the clinical characteristics of the rash in the sorivudine group, whereas there was minimal clinical deterioration of the rash in the placebo group. All 3 (100%) of the subjects in the sorivudine group who had lesions in the “moderate rash” category experienced significant clinical improvement by day 8 (i.e., the lesion either healed or was downgraded to the “mild” category), compared with 3 (60%) of 5 subjects in the placebo group. There was no difference between the 2 groups with respect to the severity of pain.

Lesion, serum, and PBMC samples obtained throughout the study were analyzed by PCR. VZV DNA was detected at the site of the lesion while vesicles were present and even after the lesions had healed. All 25 patients had viral shedding detectable by use of PCR at the site of the lesion on day 1, and 22 (88%) had detectable viral shedding on day 14. Analysis of PBMC samples indicated that viral DNA was present in 15 (60%) of 25 samples on day 1 and in 5 (20%) of samples on day 28. Lesion, serum, and PBMC samples were analyzed by use of real-time PCR (figure 2). For samples from the site of the lesion, the number of viral copies detected by real-time PCR ranged from 21,000 to 114,000,000 copies/mL on day 1 and from 21 to 14,560 copies/mL on day 14. This suggests a wide range of viral activity at the dermatome and that expression of viral DNA decreases accordingly as the disease resolves. The serum values ranged from 0 to 120 copies/mL on day 1 and from 0 to 60 copies/mL on day 14. The number of copies in PBMC samples ranged from 7 to 310 copies/mL on day 1 and from 0 to 56 copies/mL on day 8. Although the extent of systemic dissemination of the virus varies significantly among patients, all patients had detectable viral DNA in PBMCs at ≥1 time points during the study. VZV DNA was found in the serum of 76% of patients at ≥1 time points during the study. The sensitivity of quantitative real-time PCR is 1 copy of VZV DNA/mL; however, 34 or more copies of VZV DNA/mL are required for a sample to be positive by the qualitative PCR method used in this study. When this threshold was used, there was 99% agreement between the qualitative and quantitative PCR methods.

DISCUSSION

The systemic symptoms that can occur in patients with HZ, such as fever, fatigue, and malaise, suggest viremia. Prior to this study, VZV has not been consistently and systematically documented in the blood of otherwise healthy patients with shingles. In this study, we were able to detect VZV DNA in skin lesion, PBMC, and serum samples for all patients at some time point(s) during the study. Our finding that VZV DNA could be detected in 3 different sources from the same subject is unique, to our knowledge, and demonstrates that viremia definitely occurs in patients with HZ. In addition, the 2 different PCR processes, which had a 99% correlation, were performed in different laboratories by different double-blinded investigators, further strengthening the findings. The current literature contains reports of systemic evidence of VZV DNA in the serum of patients with HZ [10, 19–23]. VZV DNA has also been detected in the saliva of patients with HZ, with an association shown between the number of copies of VZV DNA and the amount of pain patients experience [11]. However, no study to date has documented consistent findings of VZV DNA from 3 separate sites (lesion swab samples, serum, and PBMCs).

In some of our subjects, viral DNA was detected in the serum at day 8 and even at day 14, and viral DNA was detected in the PBMCs at day 28 in 5 (20%) of the subjects, suggesting that the duration of viremia is longer than previously reported and that viremia persists after antiviral treatment [22, 24]. Our data showed that the duration of viremia was variable, suggesting that the extent of systemic dissemination of the virus varies significantly among patients. However, all patients had detectable viral DNA in their PBMCs during ≥1 time point during the study, and 76% of patients had detectable VZV DNA...
Figure 2. Viral load in lesion samples (A), peripheral blood mononuclear cells (B), and serum (C) obtained from patients with herpes zoster. DNA was extracted from samples and analyzed by use of real-time polymerase chain reaction for varicella-zoster virus DNA. A log transformation was used to eliminate some of the statistical skew; transformed data retained their extreme variability, which rendered parametric regression techniques inappropriate, and thus median (i.e., least absolute value) regression was used to compare median changes in the data over time.

The number of DNA copies decreased with treatment and resolution of cutaneous eruption, as seen in other studies [25]. The lesion samples were the most consistent source for the detection of viral DNA and for monitoring the decrease in copy number over the course of the disease. We did not see a correlation between the number of VZV DNA copies and pain level or between the number of VZV DNA copies and rash severity, although previous, larger studies have shown an association between viral load and prodromal pain [26]. It is possible that if our patient population had been larger, we would have seen a similar association.

Documentation and quantification of viremia in acute HZ provides a tool for monitoring treatment response in patients with HZ and for assessing the efficacy of new antiviral drugs. Furthermore, detection of viremia may allow diagnosis of HZ during the prodromal period and may allow documentation of zoster sine herpete, the syndrome of pain and paresthesia along a dermatome without the development of cutaneous findings [29].

The nucleoside analogue oral antiviral agents (acyclovir, valacyclovir, and famciclovir) are currently the standard of care for the treatment of HZ. Sorivudine is a synthetic thymidine analogue that has been formulated into a cream (ARYS-01); the drug was originally formulated in Japan for oral administration and was thought to be a promising new treatment for HZ after success in early trials in Japan and the United States [27–31]. Sorivudine was found to be a selective and potent inhibitor of VZV replication at 1 thousandth the concentration of acyclovir and to have greater oral bioavailability (of 50%–70%) than that of acyclovir (bioavailability of 15%–30%) [29, 32–35]. The drug was removed from the market in Japan and never received Food and Drug Administration approval in the United States because of multiple patient deaths that resulted from bone marrow suppression caused by the concomitant use of sorivudine and 5-fluorouracil. Bromovinyl-uracil, the principal metabolite of sorivudine, inhibits dihydropyrimidine dehydrogenase, which is the principal enzyme involved in 5-fluorouracil degradation, thus producing toxic levels of the anticancer agent in patients who take both sorivudine and 5-fluorouracil [36–38]. The topical preparation was formulated to reduce systemic absorption of sorivudine and subsequent...
formation of its metabolite, bromovinyl-uracil, while maintaining the drug’s potent activity against VZV. Sorivudine cream appears to be safe and well tolerated; however, this study found no statistical evidence for its efficacy in the treatment of HZ. Analysis of lesion, PBMC, and serum samples by use of PCR confirmed the presence of persistent viremia in patients with HZ. All sample sources showed a decrease in viral load as the disease resolved. To our knowledge, this study is the first that consistently documents viremia in a series of patients through the use of 2 different PCR technologies to analyze both serum and PBMC samples and correlates these findings with VZV DNA detected at the lesion site. VZV DNA is both cell-associated and free in the blood. Real-time PCR is more sensitive to the presence of VZV DNA and may be useful for monitoring treatment response in subjects with HZ, especially immunocompromised subjects, who have a high risk of complications. These findings reinforce the fact that HZ is actually a systemic disorder with a dermatologic manifestation. Although sorivudine 3% cream appears to be a safe and well-tolerated adjuvant to oral antivirals, further phase 2 studies are needed to determine its clinical efficacy in treatment of HZ.

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References


