The Comparative Effects of Famciclovir and Valacyclovir on Herpes Simplex Virus Type 1 Infection, Latency, and Reactivation in Mice

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Infections by herpes simplex virus (HSV) cannot yet be eliminated, but the severity of the disease can be reduced. Two newer drugs with established efficacy for such infections, famciclovir and valacyclovir, were tested in a mouse eye model of HSV infection. Both drugs significantly reduced mortality and titers of virus shed from the eyes of mice infected with an otherwise lethal dose of HSV type 1 (HSV-1). Similar titers of HSV-1 were found in the eyes, ganglia, and brains of treated animals. Although valacyclovir reduced the latent viral DNA load better in these studies than did famciclovir, rates of reactivation by explantation and UV exposure were the same. Thus, in this study, famciclovir and valacyclovir were equally effective in limiting the virulence and spread of HSV-1, despite their biochemical and pharmacologic differences.

Herpes simplex virus (HSV) types 1 and 2 are important human pathogens that cause orofacial and genital lesions [1]. During the initial infection, HSV infects neurons, where it remains in a latent state until reactivated by stimuli such as heat and UV light [2, 3]. In vivo reactivation of HSV depends on multiple factors, such as the anatomic site of infection [4], the host’s immune status [5–7], and the quantity of latent viral DNA [8].

Currently, there is no vaccine or cure for HSV infections; however, treatments are available that diminish the severity of the disease. Valacyclovir and famciclovir, the oral forms of acyclovir and penciclovir, respectively, are guanine analogues that inhibit HSV replication at the level of DNA synthesis [9–16]. Acyclovir has been the standard for treating herpes infections; however, because of its poor bioavailability (15%–20% vs. 55% for valacyclovir and 75% for famciclovir), it was prudent to develop new compounds. Following their absorption, valacyclovir and famciclovir are rapidly and efficiently metabolized by host enzymes to acyclovir and penciclovir, respectively.

Both acyclovir and penciclovir enter infected and uninfected cells but require viral thymidine kinase for activity [9, 14, 15]. Previous reports showed that penciclovir has a higher affinity than acyclovir for the viral thymidine kinase, a faster rate of phosphorylation, and a longer intracellular half-life [10, 11]; however, penciclovir has a lower affinity than acyclovir for the HSV DNA polymerase. The end result is that the penciclovir prodrug, famciclovir, which is effective in humans at doses similar to those for acyclovir, can be given less often. The higher acyclovir blood levels effected by valacyclovir achieve the same end result of reduced dosage frequency. Given these modest differences between valacyclovir and famciclovir, it is noteworthy that a series of animal studies suggested that famciclovir is better than valacyclovir in terminating ganglionic HSV infection and thereby limits subsequent reactivation [17–20].

We investigated the effects of oral famciclovir and valacyclovir on HSV-1 infection, establishment of latency, and reactivation in the mouse eye model. Our conclusions differ from those summarized above [17–20].

Materials and Methods

Cells, virus, and animals. The neurovirulent HSV-1 strain McKrae was grown in Vero (African green monkey kidney) cells in EMEM:199 medium (Quality Biological, Gaithersburg, MD) with 10% fetal bovine serum (Quality Biological) and 1% glutamine-streptomycin-penicillin (Life Technologies Gibco BRL, Gaithersburg). Hereafter we refer to this medium as EMEM medium. Female Balb/c mice, 4–6 weeks old, were obtained from the National Cancer Institute (Frederick, MD).

Inoculations and treatments. Mice were anesthetized with a 0.5-mL intraperitoneal injection of a mixture of ketamine and xylazine in PBS. Both corneas were scarified with a 25-gauge needle, and 5 μL of virus inoculum was applied per eye (total, 10⁶ pfu per mouse). Control mice received 5 μL of PBS in each scarified eye. Drug-treated animals received either famciclovir or valacyclovir (both from Midwest Medical Supply, St. Louis) ad libitum, as 1 mg/mL of drinking water beginning 24 h after injection (postinfection [pi] day 1) and continuing through pi day 7. Water bottles were changed daily to ensure fresh drug preparations.

Survival and ocular virus shedding. Mice were infected by bilateral corneal scarification and were observed daily for mortality...
Figure 1. Survival after herpes simplex virus type 1 (HSV-1) inoculation. Groups of 25 mice were infected with 10^6 pfu of HSV-1 (McKrae) by bilateral corneal scarification. 1 mg/mL of famciclovir (FCV) or valacyclovir (VACV) was administered in drinking water from 24 h (day 1) until postinfection (pi) day 7. Survival was monitored for first 15 pi days.

Figure 2. Ocular shedding after herpes simplex virus type 1 (HSV-1) infection. Groups of 25 mice were infected with HSV-1 (McKrae) and treated with oral famciclovir (FCV) or valacyclovir (VACV), as described in Figure 1. On indicated days, corneal swabs were taken for virus isolation and titration. Each point represents log value of geometric mean virus titer ± SE for 5 mice. Log values of 0 reflect undetectable virus levels.

up to pi day 15. Concurrent with survival monitoring, the quantity of virus shed from the inoculation site was determined. On the indicated days, both eyes were swabbed with a moistened Dacron polyester–tipped applicator (Baxter Healthcare, Deerfield, IL). The swab was placed in 1 mL of EMEM medium + 1.2% amphotericin B (Quality Biological). The culture material was diluted serially, and samples were plated in duplicate on Vero cells and incubated for 1 h at 37°C on a rocking platform. Afterward, cells were overlaid with medium containing 0.5% pooled human IgG (Abbott Laboratories, Abbott Park, IL) and then placed in a humidified 37°C CO2 incubator until plaques were evident. The dishes were then stained and plaques were counted.

Virus titers in tissues. On pi days 2, 4, 7, 9, and 11, mice were killed and the eyes, brain, and trigeminal ganglia (TG) were removed from infected animals by aseptic technique and placed into separate tubes containing 1 mL of medium (EMEM + 1.2% amphotericin B). Organs were ground in a tissue homogenizer (Tekmar; VWR Scientific, McGaw Park, IL), and the homogenates were diluted serially and plated in duplicate on Vero cell monolayers. Plaques were identified and counted as indicated earlier.

Explant cocultivation. Animals that survived the HSV-1 infection were allowed to develop latent infections by housing them for at least 30 days after infection. Whole TG from groups of 10 latently infected mice were removed, and each pair of TG was placed onto separate Vero cell monolayers. Explant cultures were kept in a 37°C humidified CO2 incubator. The explants were checked daily for cytopathic effects (CPEs) and carefully transferred onto fresh monolayers weekly, if necessary.

In vivo reactivation. The eyes of latently infected mice were exposed to UV radiation to reactivate HSV-1 in vivo by use of a modified procedure of Laycock et al [3]. Animals were anesthetized with a combination of ketamine/xylazine in PBS and placed onto cardboard resting on top of a 60 Hz/115 V TM-20 transilluminator (UVP, Upland, CA) emitting a peak wavelength of 302 nm. One side of the head was exposed for 1 min. Mice were then turned to expose their contralateral eye. Some infected animals were not exposed, to serve as “unstressed” controls. At 48 h after UV exposure, the pair of TG from each animal was aseptically removed and ground in 1 mL of medium (EMEM + 1.2% amphotericin B) by use of a Tekmar tissue homogenizer (VWR Scientific). The entire homogenate was plated on Vero cells and incubated at 37°C. Monolayers were checked daily for CPEs.

Quantitative polymerase chain reaction. TG pairs from latently infected mice were dissected by use of individual sterile instruments for each animal. The TG were separated and rinsed 3× each in PBS. DNA was isolated separately from each ganglion with the Puregene DNA isolation kit (Gentra Systems, Minneapolis), according to the manufacturer’s instructions. After extraction, the DNA from both TG from an animal was pooled. The total DNA recovered per TG pair averaged 7.5 μg (range, 1.5–15 μg). Purity of the DNA was checked by a ratio of the optical density (OD) readings at 260/280 nm. The number of copies of latent HSV-1 DNA was quantified by real-time fluorescence polymerase chain reaction (PCR) by use of the Taqman system (ABI Prism 7700 sequence detector; PE Applied Biosystems, Perkin-Elmer, Foster City, CA) with primers and probe specific for glycoprotein G of HSV-1 (gG-1). The reaction mixture consisted of a 1× concentration of Universal Master Mix (Perkin-Elmer), 1 μM of each primer, and 100 nM of probe. We used the sequences as follows: forward primer, CTGGTTCGTTTCTTCACCTGCT; reverse primer, CAAAGCGATAAGGTGATGAC; and 6-carboxyfluores-
Figure 3. Herpes simplex virus type 1 (HSV-1) titers in eyes, ganglia, and brain during acute infection. Mice were infected with HSV-1 (McKrae) and treated with oral famciclovir (FCV) or valacyclovir (VACV), as described in figure 1. On indicated days, eyes, pooled trigeminal ganglia, and brains were removed and titers were determined on Vero cells. Each point represents log value of geometric mean virus titer ± SE for 6 animals. Log values of 0 reflect undetectable virus levels.

Results

Survival of animals during acute HSV-1 infection. To define the effects of famciclovir and valacyclovir on virus-induced mortality, groups of 25 animals were infected by bilateral corneal scarification followed by inoculation with 10^6 pfu of HSV-1 (McKrae). Mortality was scored daily for the first 15 pi days. As shown in figure 1, 72% (18/25) of the famciclovir-treated mice survived, compared with 76% (19/25) of those treated with valacyclovir (P = .67). None of the untreated animals survived beyond pi day 5, whereas all of the mock-infected animals survived. Thus, both drugs allowed similar percentages of mice to survive an otherwise lethal inoculum of HSV-1.

Ocular shedding during acute infection. To monitor the titers of HSV-1 shed at the site of inoculation, eye swabs were taken on pi days 1, 2, 5, 7, 12, and 13. On pi day 1, all infected animals shed 5 logs of virus; the amount of shedding decreased over time (figure 2). There were no survivors in the untreated group of mice after pi day 5. Significant differences were seen on days 2 and 5 (P = .02 and .0003, respectively) between untreated and treated groups as a whole. However, there was no significant difference in the titers of virus shed from the famciclovir- and valacyclovir-treated groups (P by pi day 1, .60; 2, .39; 5, .74; 7, .84), as the resultant curves were virtually superimposable.

HSV-1 titers during acute infection. Subsequent to the initial eye infection, the virus spread to the TG and then to the brain. To study HSV-1 pathogenesis in this mouse model, we quantified the amount of virus found in the eyes, TG, and brain on pi days 2, 4, 7, 9, and 11. These tissues were removed from groups of 3 euthanized mice per treatment arm and at each time point. The experiment was done twice, and the results shown are the combined data for 6 mice per data point. As expected, there were higher tissue titers in untreated animals than in those treated (figure 3). P values were as follows for days 2, 4, and 7, respectively: eyes, <.0001, .0006, and .29; TG, .0009, <.0001, and .04; and brain—not applicable, .006, and .08. Except for 1 data point, HSV titers in the eyes, TG, and brains of famciclovir-treated mice were similar to those given valacyclovir (figure 3). P values by pi days 2, 4, 7, 9, and 11, respectively, were as follows: eyes, .18, .06, .76, .18, and .2; TG, .14, .15, .68, .04, and .45; and brain—not applicable, .74, 1.0, .88, and .17. A progression of infection was evident as virus titers in the TG peaked on pi day 4 for all 3 groups, whereas titers in the brain peaked on pi day 7. Untreated controls did not survive after pi day 7. In contrast to prior studies that used...
the ear pinna model [17, 20], we did not observe a rebound of virus titers after cessation of valacyclovir therapy.

**Explant cocultivation of latently infected ganglia.** HSV establishes a latent infection in the TG and can be reactivated in vitro by explant cocultivation. To detect any potential differences in the establishment of latency and the rate of in vitro reactivation, HSV-1±infected mice treated with famciclovir or valacyclovir were killed on pi day 44 and TG were removed. Groups of 5 (mock-infected) or 10 (drug-treated) animals were studied. Explants were cultured for 15 days, by which time compared with the ganglia of 90% of the valacyclovir-treated mice (table 1). As expected, no virus reactivated from mock-infected/total tested or in the average time to reactivation between the famciclovir and valacyclovir groups (9.7 vs. 10.6 days, respectively). No virus was recovered from UV-treated, mock-infected mice. To ensure that the reactivation was due solely to UV stress, we homogenized TG from latently infected but unstressed animals: none of these animals yielded reactivated virus.

**Quantitative PCR to determine latent virus load in TG.** As a means of determining the amount of latent HSV present in the TG, we developed a novel, real-time quantitative PCR assay based on displacement of a fluorescently labeled probe to HSV-1 gG-1 sequences. Groups of 8 HSV-1 latently infected animals were killed on pi day 73, and individual ganglia were removed for DNA extraction and subsequent PCR. By comparing the PCR results to a standard curve run simultaneously of serial dilutions of a gG-1 plasmid (figure 4), we estimated the genome copy number in test samples. We found slightly, but significantly, more copies of latent HSV-1 in the famciclovir-treated mice compared with those given valacyclovir (P = .003; figure 5). There was an average of 3.6 logs of latent virus per 145 ng of mouse TG DNA in the famciclovir-treated group (95% confidence interval [CI], 3.5–3.7 logs) vs. 2.9 logs in the valacyclovir-treated group (95% CI, 2.8–3.0 logs).

### Table 1. Reactivation of latent herpes simplex virus type 1 by two methods.

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of ganglion pairs reactivating/total tested</th>
<th>Mean days to reactivationa</th>
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<tbody>
<tr>
<td>Mock infected</td>
<td>0/5</td>
<td>N/A</td>
</tr>
<tr>
<td>FCV</td>
<td>10/10</td>
<td>9.7 ± 0.2</td>
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<tr>
<td>VACV</td>
<td>9/10</td>
<td>10.6 ± 0.7</td>
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<tr>
<td>UV irradiation</td>
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<tr>
<td>Mock infected</td>
<td>0/5</td>
<td>N/A</td>
</tr>
<tr>
<td>FCV</td>
<td>5/9</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>VACV</td>
<td>7/10</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Mock infected, no UV</td>
<td>0/5</td>
<td>N/A</td>
</tr>
<tr>
<td>FCV, no UV</td>
<td>0/10</td>
<td>N/A</td>
</tr>
<tr>
<td>VACV, no UV</td>
<td>0/10</td>
<td>N/A</td>
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NOTE: FCV, famciclovir; VACV, valacyclovir; N/A, not applicable.

a ± SEM.

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**Discussion**

The severity of HSV infections can be mitigated by antiviral chemotherapy [14, 16, 23–26]. Two newer drugs with established efficacy for such infections, famciclovir and valacyclovir, proved in this study to be equally effective at limiting the virulence and spread of HSV-1, despite their biochemical and pharmacologic differences. In humans, there is 55% bioavailability of acyclovir from valacyclovir and 75% penciclovir from famciclovir [14, 15]. In addition, the maximum serum levels are similar for penciclovir and acyclovir, although the time to reach these levels was faster for penciclovir (reviewed elsewhere [15]). In cells, penciclovir has a higher affinity than acyclovir for the viral thymidine kinase, a faster rate of phosphorylation, and a longer half-life [10, 11, 15]; however, penciclovir has a lower affinity for the HSV DNA polymerase. Despite these pharmacokinetic distinctions between the two drugs, no in vivo differences were observed in this study. Both drugs significantly reduced the mortality and titers of virus shed from the eyes of mice infected with an otherwise lethal dose of HSV-1 (figures 4 and 5).
Figure 5. Quantitation of latent herpes simplex virus type 1 (HSV-1) DNA copy numbers by polymerase chain reaction (PCR). On postinfection day 73, groups of 8 mice were killed and individual ganglia were removed for DNA extraction. Each point represents log value of geometric mean copy number for 6 replicate PCR determinations per pooled trigeminal ganglia from each animal. Horizontal line (along width of diamond) indicates combined mean of HSV-1 DNA copy nos. for all 8 mice/group; shorter horizontal lines indicate 95% confidence intervals around mean. FCV, famciclovir; VACV, valacyclovir.

1, 2). In addition, we found similar titers of HSV-1 in the eyes, ganglia, and brains of the treated animals and no rebound of virus titer after termination of valacyclovir therapy (figure 3), unlike results with a different mouse model, as described elsewhere [17, 20].

Neither famciclovir nor valacyclovir prevented the establishment and maintenance of latency, as evidenced by reactivation and quantitative DNA PCR studies. The rates and proportions of viral reactivation induced by explant cocultivation of whole TG and by exposure to UV light were similar (table 1). Other reports suggest that the latent viral DNA load determines the rate of reactivation and disease [8, 27–29]. Given our observations of similar rates of reactivation, we expected to find equivalent copy numbers of latent HSV DNA in the ganglia. While valacyclovir reduced the latent viral DNA load slightly better in these studies than famciclovir (this was the only difference found in the entire study; figure 5), rates of reactivation by explantation and UV exposure were the same. This result may reflect the fact that we induced viral reactivation, both in vitro and in vivo, with very powerful and efficient stimuli. If one could study spontaneous viral reactivation in mice, one might see differences in reactivation rates as a function of latent viral DNA load.

We used strain McKrae instead of the strains (i.e., SC16, F) used by others [17, 19, 20], because of its high neurovirulence compared with strains passaged multiple times in the laboratory, which are consequently more attenuated. Because of its virulence, we believe that McKrae-induced infection and disease closely resemble that seen in humans. Animals were infected at the virus dose chosen on the basis of pilot studies that showed that to study latency and reactivation, a sufficient quantity of input virus is needed. Recent observations in our laboratory, however, suggest there may be a threshold level of virus load, above which further increments in reactivation rates no longer occur (M. Godleski and K. Wang, unpublished data), and that the threshold may have been attained with the high virus inoculum that we used. Given the virulence of HSV-1 McKrae, the inoculum required to reproducibly yield reactivating virus in the face of antiviral treatment proved 100% fatal in the absence of treatment.

Despite these methodologic issues, the cumulative results of the present study are consistent with most laboratory and clinical assessments of famciclovir and valacyclovir [9–16]. They differ notably from the serial comparative mouse studies of famciclovir and valacyclovir by Thackray, Field, and et al. [17–20], who observed the aforementioned rebound in virus titers after conclusion of valacyclovir treatment. Of importance, they detected a greater reduction in latent HSV-1 in famciclovir-treated animals, as reflected in lower rates of explant reactivation and fewer neurons expressing viral latency–associated transcripts. The differences highlighted between those studies and the present one are few, but they may underlie the distinct results. Thackray and Field infected mice with strain SC16 by the ear pinna route in which virus ascends to the ganglia and then descends to induce an acute zosteriform eruption. It is possible that the regional cutaneous spread of HSV in this model provides an extended period in which ganglia are seeded with virus, during which one antiviral agent might successfully intercede better than the other. We are confident, however, that our in vivo reactivation and PCR studies more accurately reflect levels of latent virus than the methods used in those earlier experiments. As such, they cast considerable doubt as to the ability of one of these two newer antiviral drugs to better alleviate latency and subsequent reactivation than the other, even if administered early in the course of primary infection.

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References

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