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Lack of effect of treatment with penciclovir or acyclovir on the establishment of latent HSV-1 in primary sensory neurons in culture

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Abstract

Recent studies suggest reductions in establishment of herpes simplex virus, type 1 (HSV-1) latency using the nucleoside analog penciclovir compared with acyclovir in the murine model. These observations raise the possibility that the new analogs may have novel activities that directly interfere with the establishment of the latent infection, suggesting a mechanism other than simply blocking the productive infection. To determine if penciclovir has a direct action on the establishment of latency, we compared the effects of penciclovir versus acyclovir in an in vitro model of HSV-1 latency in rat dorsal root ganglia neurons in culture. In neurons in culture, both penciclovir and acyclovir were highly effective in blocking the productive infection. However, neither penciclovir nor acyclovir blocked establishment of latency as demonstrated by similar percentages of neurons expressing the latency-associated transcript (LAT). Following removal of the respective nucleoside analog, latency was maintained until reactivation was induced by nerve growth factor deprivation. Similar virus titers were recovered after induction of reactivation of latent infections, which were established in the presence of either penciclovir or acyclovir. These results indicate that neither penciclovir nor acyclovir treatment directly prevents the establishment of latent HSV-1 infections in primary sensory neurons in culture. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Herpes simplex virus; Latent infection; Reactivation; Neuron; Acyclovir; Penciclovir

A major goal in the treatment of human herpes simplex virus (HSV) infections is to develop therapies that will interfere with the establishment of the latent HSV infection. This would prevent the diseases associated with HSV and prevent human to human transmission of the virus. The clinical introduction of nucleoside analogs, such as acyclovir, has correlated with a major shift in the morbidity and mortality associated with serious HSV infection. However, significant neurologic sequela remain the outcome of herpes encephalitis caused by HSV-1 and in the neonatal infections caused by HSV-2 (Overall, 1994; Jacobs, 1998). In

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addition, there is no evidence that these drugs can alter the latent HSV-1 infection once it has been established.

Separating potential drug effects on the productive infection from effects on the establishment of latency can be relatively difficult, since agents that interfere with the productive infection may indirectly result in the decreased establishment of latency by reducing the amount of virus that reaches the neuron. The nucleoside analogs share a common requirement for the viral encoded thymidine kinase (TK) for conversion to an active form, although the affinity for the active site of the enzyme varies considerably (Darby, 1994; Champness et al., 1998). In experimental models of HSV-1 latency, nucleoside analogs, such as acyclovir, given during the primary infection reduce the amount of latent HSV-1 in the ganglia during the latent infection (Klein et al., 1979; Field and De Clercq, 1981; Klein et al., 1983; De Clercq, 1993; Sawtell, 1997; Thackray and Field, 1996; LeBlanc et al., 1999a; Thackray and Field, 1998). However, delay of initiation of drug treatment does not alter viral latency even when extended treatment periods are utilized (Blyth et al., 1980; Field and De Clercq, 1981). These data are consistent with a model for the establishment of HSV-1 latency that requires little or no viral gene expression in the neuron for the establishment of HSV-1 latency.

Unexpected results were obtained in recent studies of the effects of antivirals with improved bioavailability, famciclovir and valaciclovir, which are oral pro-drug forms of the active drugs, penciclovir and acyclovir, respectively (Field et al., 1995; Thackray and Field, 1998, 2000). In these studies, famciclovir was shown to be significantly better in reducing establishment of latency and was effective even when initiation of drug treatment was delayed during the primary infection. As observed with other nucleoside analogs, famciclovir treatment did not affect the latent infection once latency was established. In contrast, however, in other studies different effects of treatment with famciclovir compared with valacylovir on reactivation were not observed (LeBlanc et al., 1999b).

As a direct approach to compare the effects of the nucleoside analogs on the establishment of latency, we used a model of HSV-1 latency in dorsal root ganglia neurons in culture (Wilcox et al., 1990; Smith and Wilcox, 1996). In this system latency is established without a productive infection and quantitative measurements of the efficiency of the establishment of latency are readily obtained (Wilcox et al., 1997). This allowed the direct comparison of the effects of treatment with penciclovir versus acyclovir on the establishment of the latent HSV-1 infection.

In the mouse model, one potential mechanism of action of nucleoside analogs that affects the establishment of latency is to reduce or prevent the productive infection that occurs at the site of inoculation or in the ganglia. The use of the in vitro neuronal HSV-1 latency model allows manipulation of the multiplicity of infection, which determines whether either a synchronized lytic or latent infection occurs (Smith et al., 1994). To compare the effects of the nucleoside analogs on the productive infection, neuronal cultures were infected with ~ 100 pfu of HSV-1 per neuron with or without drug treatment, and viral titers were determined (Fig. 1). In the presence of either 50 mM penciclovir or acyclovir, virus replication was significantly suppressed. However, complete destruction of essentially all of the neurons was observed by 3 days postinfection whether either of the drugs was present or absent. These results indicate that viral cytotoxicity was not prevented by drug treatment, even though both drugs were highly effective in preventing the replication of virus.

We have previously shown that neurons in culture can be infected with HSV-1 under conditions that lead to the very efficient establishment of latency (Wilcox and Johnson, 1987, 1988; Wilcox et al., 1990, 1992; Smith et al., 1994). Latently infected neuronal cultures were prepared essentially as previously described, with the modification of the use of penciclovir to compare with acyclovir treatment during the establishment of latency (Wilcox and Johnson, 1988; Wilcox et al., 1990). Fourteen days after plating the neuronal cultures, 50 mM penciclovir or acyclovir was added to the culture media 12 h prior to and for

7 days following inoculation with HSV-1. The neuronal cultures were inoculated with a multiplicity of infection of ~10 pfu per neuron. Seven days after virus inoculation, the culture medium was replaced with standard culture medium, which lacks an antiviral agent. Fourteen days after virus inoculation, the neuronal cultures were used in the experiments described. The standard neuronal culture medium consisted of 10% newborn bovine serum (Gibco, Grand Island, NY) and 100 ng/ml of 2.5 S mouse nerve growth factor (Harlan Bioproducts) in Dulbecco's Minimum Essential Medium (Gibco/BRL).

During the latent HSV-1 infection, only one region of the viral genome is abundantly transcribed, termed the latency-associated transcript (LAT) (Fraser et al., 1992; Wagner and Bloom, 1997). As one measure of the latent infection, the percentage of LAT-positive neurons was determined by in situ hybridization. Neuronal cultures were examined after HSV-1 latency was established in the presence of penciclovir or acylovir. Significant cell loss did not occur in cultures treated with either penciclovir or acyclovir based on cell counts compared to mock-infected controls. Representative views of neuronal cultures harboring the latent virus had identical patterns of nuclear expression of the LAT in cultures established in either the presence of penciclovir or acyclovir (Fig. 2). The percentage of neurons expressing LAT during the latent infection was virtually identical for latency established in the presence of penciclovir or acyclovir (Fig. 3). These data show that neither penciclovir nor acyclovir treatment prevented the establishment of the latent infection. These results indicate that when latency was established under conditions that produce high percentages of neurons harboring latent HSV-1, neither of the nucleoside analogs had effects that could explain the differences observed in vivo. While the detection of LAT may underrepresent the number of neurons that harbor latent HSV-1, previous studies have shown that detection of LAT has a high correlation with the amount of viral DNA present in the in vitro neuronal model of HSV-1 latency (Wilcox et al., 1997).

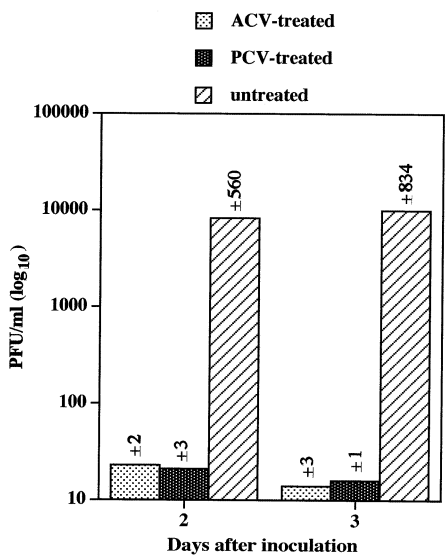

Fig. 1. Comparison of effects of acyclovir or penciclovir treatment on the productive HSV-1 infection in neurons. Neuronal cultures were prepared from the dorsal root ganglia (DRG) of embryonic day 15 rats as previously described (Smith et al., 1994; Wilcox et al., 1992; Wilcox and Johnson, 1988, 1987; Wilcox et al., 1990). HSV-1 (17^+), which was prepared and quantified by plaque-formation assay on Vero cells (ATCC), was used at a MOI of 100 pfu per neuron. The cultures were harvested at 2 or 3 days after inoculation and titers determined. The bars indicate the mean titers for each treatment group with the indicated SEM ($n = 5$). Similar results were obtained in three separate experiments.

Most evidence suggests that the nucleoside analogs have no effects on virus that has achieved latency (Blyth et al., 1980; Field and De Clercq, 1981). However, it remains possible that drugs present during the establishment of latency could result in destabilization or an alteration in the latent genome or virus-neuron interactions that could potentially reduce the ability of the latent virus to reactivate. However, the similarity of mechanism of action and the shared requirement for viral TK for activation of the nucleoside analogs suggests that these agents would not affect the latent HSV-1 infection by direct action on virus that has reached the latent state. However, there is evidence of limited expression from the region of the TK gene in the mouse model of HSV-1 latency (Kosz-Vnenchak et al., 1993; Kramer and Coen, 1995), suggesting a more active state of the viral genome during the latent

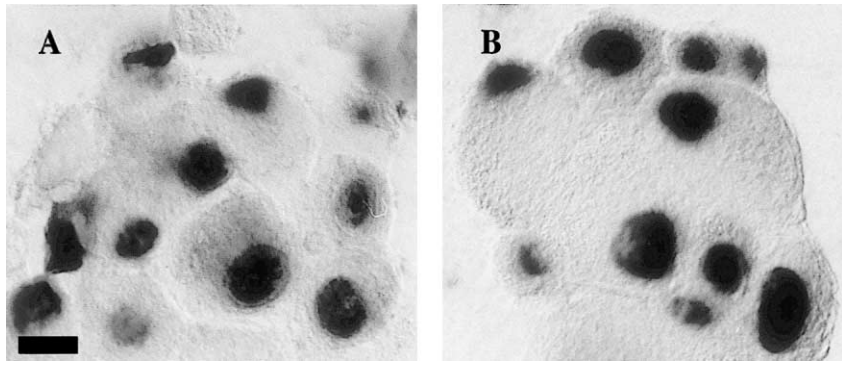

Fig. 2. Comparison of effects of penciclovir versus acyclovir treatment during the establishment of latency on LAT expression during the latent HSV-1 infection in neuronal cultures. Neuronal cultures were prepared and HSV-1 latency was established as previously described (Wilcox and Johnson, 1987, 1988; Wilcox et al., 1990, 1992; Smith et al., 1994). LAT was detected by in situ hybridization as previously described (Wilcox and Johnson, 1987, 1988; Wilcox et al., 1990, 1992; Smith et al., 1994). In uninfected control cultures and with the sense, control riboprobe no signal was detected (data not shown). Representative results are shown. (A) Neuronal cultures treated with 50 μM penciclovir during the establishment of latency. (B) Neuronal cultures treated with 50 μM acyclovir during the establishment of latency. Bar in (A) is 10 μm and also applies to (B). Similar results were obtained in three separate experiments.

infection that would potentially be affected by the nucleotide analogs.

The effect of treatment with the nucleoside analogs during the establishment of latency on the subsequent ability of the latent virus to reactivate in the neuronal cultures was examined. Neuronal cultures harboring latent HSV-1 were induced to reactivate by nerve growth factor withdrawal as previously described (Wilcox and Johnson, 1988; Wilcox et al., 1990). At times after the induction of reactivation, neuronal cultures were harvested and tested for the presence of infectious virus (Fig. 4). The kinetics of reactivation and the amount of virus present at the times after induction of reactivation were essentially identical whether the cultures had previously been treated with penciclovir or acyclovir. The presence of either penciclovir or acyclovir during the establishment of latency had no significant differential effect on reactivation of the latent HSV-1, supporting the LAT in situ results showing similar efficiency in the establishment of latency in the presence of either penciclovir or acyclovir.

Studies of HSV-1 in a murine model of HSV-1 latency demonstrate superior actions of orally available forms of penciclovir compared to acyclovir, both in preventing latency during the primary infection and in providing a longer time period for effective initiation of treatment during the primary infection. These findings suggest that penciclovir may have different effects on establishment of latency rather than acting only on the productive infection. The complexity of the HSV-1 infection in vivo limits the ability to determine if the observed differences in the drug effects are the result of actions on the productive infection, direct effects on interaction of the virus with the neuron, or effects the latent infection. Productive HSV-1 infections, which occur both at the site of inoculation and in the ganglia, clearly have great potential to affect the efficiency of the establishment of latency. If virus replicates to higher titers and spreads more in the skin or within the ganglia, there is greater interaction of virus with more neurons, which may lead to more latently infected neurons. There is considerable temporal overlap in the productive infection and the establishment of the latent state in vivo. To provide an alternative approach for examining these issues, the affects of the nucleoside analogs were examined in a model of HSV-1 latency in primary sensory neurons in culture. This model allowed controlled drug delivery. In addition, latent infections were established in the absence of a productive infection. In this model, treatment with penciclovir was no more effective than acyclovir in blocking the establishment of the latent HSV-1 infection.

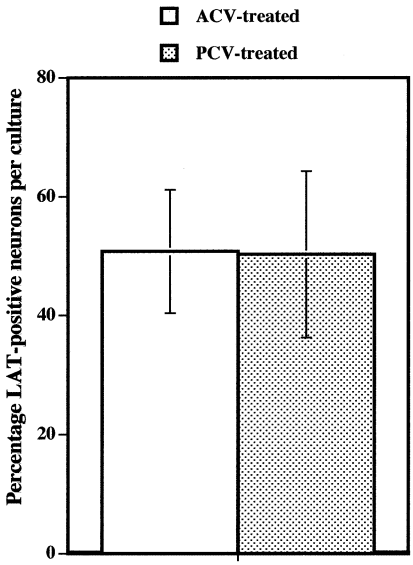

Fig. 3. Percentage of LAT-positive neurons detected using in situ hybridization. Cultures were treated with either penciclovir or acyclovir during the establishment of latency and LAT expression was detected as described in the legend for Fig. 2. The frequency of LAT-positive neurons was determined from neuronal cell counts of five random fields (containing 20–50 neurons each) per culture from six cultures, using a magnification of 200 × for the counting. Values shown are the mean ± SEM ($n = 6$). Similar results were observed in three separate experiments.

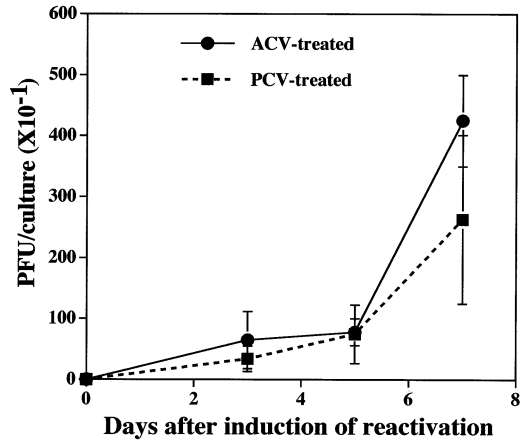

Fig. 4. Reactivation of latent HSV-1 in neuronal cultures treated with either acyclovir or penciclovir during the establishment of latency. Neuronal cultures harboring latent HSV-1were induced to reactivate by nerve growth factor withdrawal as previously described (Wilcox and Johnson, 1987, 1988; Wilcox et al., 1990, 1992; Smith et al., 1994). The cultures were harvested and tested for the presence of infectious virus in plaque-formation assays using total culture lysates, prepared by three freeze–thaw cycles, titered on Vero indicator cells. In the untreated latently infected cultures, no infectious virus was detected at any of the time points. Values shown are the mean ± SEM ($n = 5$). Similar results were obtained in three separate experiments.

These studies indicate that the differences observed the mouse model leading to the enhanced effectiveness of penciclovir over acyclovir in reducing latent HSV-1 infections do not appear to involve direct interactions between the virus and the neuron. It is more likely that biochemical or pharmacological differences between the drugs are responsible for the results in vivo. In vivo neither drug is able to completely prevent the establishment of latent HSV-1 infections. The results in the neuronal cultures are also consistent with the general lack of effect of nucleotide analogs once latency has been established. The results from the in vitro neuronal model suggest that once the virus has access to the neuron, it can establish a latent infection without replication and that the establishment of the latent infetion is not affected by treatment with the nucleoside analogs.

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