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Comparative efficacy of acyclovir and vidarabine on the replication of varicella-zoster virus

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Abstract

Acyclovir and less frequently, vidarabine are (or have been) used in the treatment of varicella-zoster virus (VZV) infection and are administered either intravenously (vidarabine) or orally (acyclovir, up to five times per day). The pharmacological bases of the administration interval were modeled in vitro in this study. Incubation of VZV-infected cultures with acyclovir or vidarabine for 24, 48, 72 and 96 h showed similar duration-dependent anti-viral activities as assessed by a plaque-reduction assay. Treatment with vidarabine for only 8 h/day for 4 days (intermittent treatment) showed anti-VZV activity equivalent to that of continuous treatment for 4 days in terms of the inhibitory dose that reduced plaque formation by 50% (IC_{50}). In contrast, intermittent treatment with acyclovir exhibited a 7.9 times higher IC_{50} value than that of continuous treatment. The mode of inhibition of expression of most of viral protein was similar in both drugs, but the degree of inhibition was different for each protein. Thus, vidarabine with a limited period of treatment showed anti-VZV activity comparable to continuous treatment with acyclovir, indicating the longer duration of anti-viral activity of vidarabine.

Keywords: Vidarabine; Acyclovir; Varicella-zoster virus; Infection; Drug resistance

1. Introduction

Acyclovir and vidarabine [arabinosyl adenine (ara-A)] has been used in the treatment of varicella-zoster virus (VZV) infection. Acyclovir is an anti-viral nucleoside analogue that exhibits anti-viral activity through phosphorylation by thymidine kinase (TK) and inhibition of viral DNA synthesis by competitive inhibition with guanosine triphosphate and chain termination after incorporation of acyclovir triphosphate (as the monophosphate) into DNA (Biron and Elion, 1980; Elion et al., 1977). Moreover, the effect of incorporation of acyclovir monophosphate into the growing DNA results in a blockage similar to suicide substrate inhibition (Reardon and Spector, 1989). Conventional acyclovir treatment rarely generate acyclovir-resistant viruses in immunocompetent hosts. However, acyclovir-resistant mutants have been isolated in immunocompromised patients upon prolonged acyclovir treatment. Currently TK-negative mutants have been rarely observed in herpes simplex virus (HSV)- and VZV-infected patients. The other mechanism of resistance involves DNA polymerase mutants (Coen and Schaffer, 1980; Collins et al., 1989; Field et al., 1980; Furman et al., 1981; Kamiyama et al., 2001; Knopf et al., 1981; Shiraki et al., 1990).

Vidarabine inhibits viral DNA synthesis at concentrations below those required to inhibit host cell DNA synthesis (Shipman et al., 1976) and may have multiple sites of action within an infected cell. It is phosphorylated to its active triphosphate form by cellular kinases rather than by a virus-induced thymidine kinase (Schwartz et al., 1984). Thus, vidarabine is capable of inhibiting TK-deficient mutants of HSV and VZV that are resistant to acyclovir (Larder and

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Darby, 1986; Shiraki et al., 1990). Further, we have reported that acyclovir-resistant DNA polymerase mutants of VZV show two patterns of susceptibility to vidarabine; one group of acyclovir-resistant DNA polymerase mutants is more sensitive to vidarabine than the parent wild-type virus and the other group showed two or three times higher 50% inhibitory concentration (IC_{50}) values than the parent wild-type virus (Kamiyama et al., 2001). This study clearly showed that vidarabine acts on viral DNA polymerase and that both types of acyclovir-resistant DNA polymerase mutants are sensitive to vidarabine. Thus, vidarabine is useful for all types of acyclovir-resistant mutants with TK mutations and DNA polymerase alterations.

The clinical efficacy of acyclovir and vidarabine has been directly compared in immunocompromised patients with varicella-zoster virus infection. Past studies have reported that acyclovir is equal to or more effective than vidarabine, and that vidarabine shows greater toxicity than acyclovir (Shepp et al., 1986; Whitley et al., 1992). However, vidarabine may still be an important drug because acyclovir-resistant mutants show clinical sensitivity to vidarabine (Reusser et al., 1996) as expected from the sensitivity in vitro. Acyclovir and vidarabine are intravenously administered three times a day and once a day, respectively. There are some studies demonstrating pharmacologic basis for administration interval in HSV-infected cells (Vere Hodge, 1993; Schwartz et al., 1984), but this is not clear in VZV-infected cells.

In this study, we investigated the anti-VZV activity and effects of acyclovir or vidarabine on viral replication and viral protein synthesis and observed that intermittent vidarabine blocked VZV replication almost as efficiently as continuous vidarabine or acyclovir. Thus, these results contribute to the pharmacological bases for the clinical use of these drugs.

2. Materials and methods

2.1. Virus

Wild- type VZV Oka strain was propagated in human embryonic lung (HEL) cells and cell-free virus prepared as described previously (Shiraki et al., 1982, 1990; Shiraki and Hyman, 1987). Briefly, infected cells were harvested and suspended in SPGC medium [phosphate-buffered saline containing 0.1% sodium glutamate, 5% sucrose, 10% fetal bovine serum (FBS)] and then sonicated, followed by centrifugation. The supernatant was frozen at −85 °C as the cell-free virus stock until use.

2.2. Cells

HEL cells were grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 10 or 2% heat-inactivated FBS.

2.3. Compounds

Acyclovir was purchased from Sigma, MO, and vidarabine was provided by Mochida Pharmaceutical Co., Japan. The drugs were dissolved in dimethylsulfoxide at 10 mg/ml and used in a plaque-reduction assay.

2.4. Plaque-reduction assay

The anti-VZV activity of acyclovir and vidarabine was examined by the plaque-reduction assay (Shiraki et al., 1982, 1990). All assays were carried out in confluent HEL cell monolayers in 60-mm plastic dishes. The cells were infected with 100 plaque-forming units/0.2 ml of cell-free VZV for 1 h. Thereafter, the cells were exposed to various concentrations of the anti-viral compounds (0.5, 1, 2, 5, 10, 20, 50 and 100 μg/ml) in MEM supplemented with 2% FBS, and incubated at 37 °C as indicated below.

The first part of the experiment was designed to compare the anti-VZV efficacy of continuous and intermittent treatment with acyclovir and vidarabine. Infected cultures were treated with acyclovir or vidarabine continuously for 4 days to determine the conventional IC_{50}. Other infected cultures were incubated in parallel, first for 8 h with the drug and then, 16 h without the drug every 4 days. After the drug-containing medium was removed, the cultures were washed by the drug-free medium once, and then was added new drug-free medium and the cells were incubated again. After 4 days, the cells were fixed with 5% neutral formalin and stained with 0.03% methylene blue. Four independent experiments were performed. The number of plaques was counted under a dissecting microscope, and the IC_{50} value was determined by interpolation from linear regression of a semi-log plot (nine data points).

The second part of the experiment was designed to compare the duration of treatment necessary to exhibit anti-VZV activity. The infected cells were incubated with the drug for first 12, 24, 48 and 96 h and thereafter incubated without any drug for 84, 72, 36 and 0 h until fixation and staining. All cultures were incubated for a total of 96 h.

2.5. Effects of acyclovir and vidarabine on viral protein synthesis

The effects of acyclovir and vidarabine on viral protein synthesis were examined in HEL cells infected with the VZV Oka strain (Shiraki et al., 1982; Shiraki and Hyman, 1987). The first part of the labeling experiment was designed to investigate the time-course profile of inhibition of viral protein synthesis by the drugs. HEL cells were infected with VZV (Oka strain). When the cytopathic effect appeared (72 h after infection), 50 μg/ml acyclovir or vidarabine was added to each medium, and the cells were further incubated at 37 °C. Then, VZV-infected cells were labeled with [^{35}S] methionine and cysteine (37 Tbq/mmol, Amersham

Biosciences, Piscataway, NJ) for 5–8, 21–24 and 45–48 h in the presence of acyclovir or vidarabine after starting drug treatment.

The second part of the labeling experiment was designed to compare the dose-dependent efficacy of the drugs on viral protein synthesis. VZV-infected HEL cells exhibiting cytopathic effects were treated with medium containing various concentrations of acyclovir (5–100 μg/ml) or vidarabine (10–300 μg/ml), and incubated at 37 °C. VZV-infected cells were labeled with [^{35}S] methionine and cysteine (37 Tbq/mmol, Amersham Biosciences) for 21–24 h after addition of acyclovir or vidarabine. The labeled cells were lysed, and viral proteins were immunoprecipitated with immunoglobulin for human use (Miles Inc., Elkart, IN) (Kurokawa et al., 1995). The immunoprecipitates were subjected to SDS–polyacrylamide gel electrophoresis followed by fluorography (Kurokawa et al., 1990, 1995; Shiraki et al., 1982; Shiraki and Hyman, 1987). Protein bands on the exposed films were scanned with a Bio-Rad Image Analysis System with a Fluor-S^{TM} MultiImager and quantitatively analyzed by the Macintosh software Multi-Analyst 1.0.

3. Results

3.1. Comparison of continuous and intermittent treatment

Table 1 compares the IC_{50} values of continuous and intermittent treatment with acyclovir and vidarabine on anti-VZV activity as assessed by the plaque-reduction assay. Intermittent treatment with vidarabine was almost equivalent to continuous treatment, and the inhibitory IC_{50} were 2.5 and 1.6 μg/ml, respectively. In contrast, the IC_{50} of intermittent treatment and continuous treatment with acyclovir were 14 and 1.8 μg/ml, respectively, and thus, the IC_{50} of intermittent treatment with acyclovir was 7.9 times larger than that of continuous treatment.

Table 1
Comparison of continuous and intermittent treatment with acyclovir and vidarabine in terms of anti-VZV activity

	IC_{50} (μg/ml) ± S.D.	
	Acyclovir, $p < 0.05$	Vidarabine, $p = 0.19$
Continuous	1.8 ± 0.1	1.6 ± 0.4
Intermittent	14 ± 1.0	2.5 ± 1.2

The anti-VZV activities of acyclovir and vidarabine in the plaque-reduction assay were compared by two different treatments. The infected cells were treated with drugs at concentrations of 0, 0.5, 1, 2, 5, 10, 20, 50 and 100 μg/ml continuously for 4 days (continuous) or for only 8 h/day for 4 days (intermittent). The number of plaques was counted under a dissecting microscope, and IC_{50} values were calculated. Student's t-test was used in the statistical analysis. *p* values indicate the comparison between continuous and intermittent treatment.

3.2. Effect of treatment period on plaque reduction

The results of treatment with acyclovir or vidarabine for 12, 24, 48, and 96 h are shown in Fig. 1. Both acyclovir and vidarabine showed similar duration-dependent anti-viral activity. The IC_{50} value curves of each treatment are shown in Fig. 2. The IC_{50} values of acyclovir were higher in treatment for 12 and 24 h than those of vidarabine. On the other hand, the IC_{50} of acyclovir and vidarabine were almost equivalent with the 48- and 96-h treatment periods. There was a large difference in IC_{50} between acyclovir treatment for 24 and 48 h. The IC_{50} of acyclovir treatment for 24 h was 7.1 times higher than that for 48 h, while the IC_{50} of vidarabine at 24 h was 2.8 times higher than that at 48 h. Thus, vidarabine treatment for a short period showed stronger anti-viral activity than acyclovir treatment.

3.3. Inhibitory effect of drug on viral protein synthesis

Synthesis of VZV proteins was compared in the presence of acyclovir and vidarabine with various treatment periods (Fig. 3a). The intensity of most of viral protein bands became weaker time-dependently following treatment with both acyclovir and vidarabine. Major protein bands on autoradiograms (175, 155, 130, 118, and 100 kDa proteins)

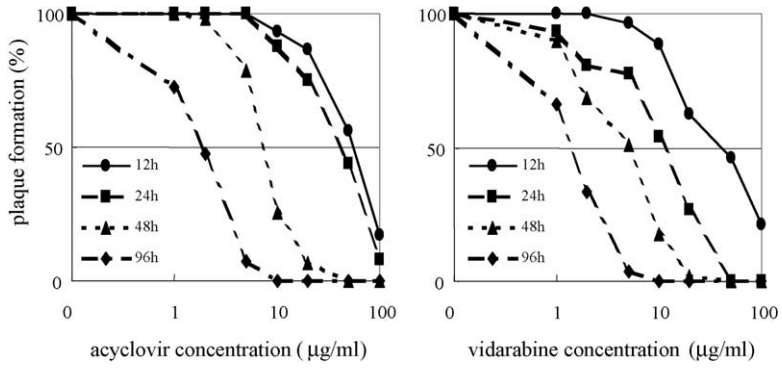

Fig. 1. Comparison of time-dependent anti-VZV activities of acyclovir and vidarabine. The infected cells were first treated with the drug at concentrations of 0, 0.5, 1, 2, 5, 10, 20, 50 and 100 μg/ml for 12, 24, 48 or 96 h and thereafter incubated without any drug. All cultures were fixed after total 96 h (4 days) incubation.

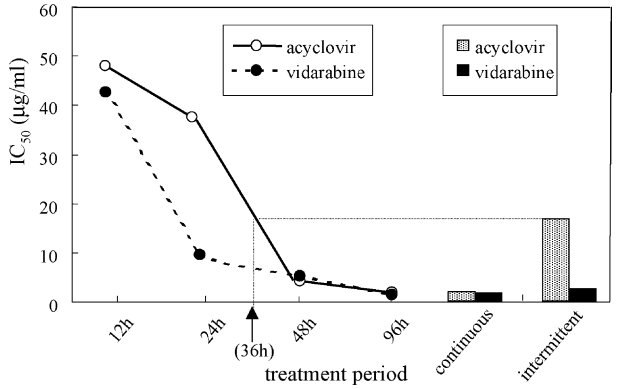

Fig. 2. Comparison of IC_{50} values upon treatment with acyclovir and vidarabine for different time periods. The IC_{50} was determined graphically from the results of the plaque-reduction assay shown in Table 1 and Fig. 1. The IC_{50} value of intermittent acyclovir treatment (total treatment time, 8 h/day × 4 days = 32 h) was almost comparable to 36 h acyclovir treatment as shown in the graph.

Table 2
Comparison of inhibition of main VZV proteins treated with acyclovir and vidarabine

VZV protein (kDA)	Acyclovir	Vidarabine
175	2.5	5.0
155	3.1	5.0
130	4.3	5.9
118	5.0	5.5
100	25.7	9.0

Percent intensity of the bands in Fig. 4 was determined from the intensities of the bands in the absence of acyclovir and vidarabine as a standard, and the IC_{50} values (μg/ml) of main VZV proteins were calculated.

were analyzed quantitatively by an Image Analysis System for the amounts of proteins synthesized. Fig. 3b shows the percent density of each protein band for untreated cells and drug-treated cells. Synthesis of most of viral protein was inhibited time-dependently by acyclovir. The 175 kDa protein was inhibited most strongly among the five viral proteins, and the 100 kDa protein was inhibited most weakly in acyclovir-treated cultures. The same tendency toward inhibition of viral protein synthesis was observed in vidarabine-treated cells. Synthesis of VZV proteins was barely detected in cells treated with vidarabine for 48 h.

3.4. Effect of drug concentration on viral protein synthesis

Synthesis of VZV proteins was compared using various drug concentrations, as shown in Fig. 4. The pattern of protein synthesis was similar in cultures treated with both acyclovir and vidarabine. The IC_{50} values are presented in Table 2. The synthesis of each protein was inhibited dose-dependently up to 10 μg/ml of acyclovir and vidarabine, and the pattern was not influenced by drug concentrations of 30 μg/ml and more of both drugs. This indicated that the inhibitory activity of acyclovir and vidarabine was saturated by treatment for 48 h, even at low concentrations.

The inhibition patterns of protein synthesis were similar in cells treated with acyclovir and vidarabine, as shown in Fig. 3b. The degree of each viral protein inhibition was equal under the condition of the long-term (24 h) treatment time in this setting.

4. Discussion

Placebo-controlled trials have shown that vidarabine and acyclovir are beneficial in the treatment of varicella-zoster virus infections in immunocompromised patients (Whitley et al., 1976, 1982) and herpes simplex virus infections in immunocompromised patients (Whitley et al., 1984). Subsequent trials, however, demonstrated that acyclovir is more effective and/or less toxic for these conditions (Whitley et al., 1992). Intravenous acyclovir has been considered most effective in preventing visceral complications of varicella-zoster virus infection in immunocompromised patients with disseminated cutaneous zoster. The most common adverse effects of intravenous vidarabine have been gastrointestinal (nausea, vomiting and diarrhea) and neurologic (tremors, paresthesia, ataxia and seizures) (Ross and Balakrishnan, 1976; Sacks et al., 1979).

The mechanism of vidarabine is not well understood. The mechanisms proposed to explain the inhibition of viral replication by vidarabine include: (a) selective inhibition of the viral DNA polymerase by vidarabine triphosphate due to competitive inhibition with the normal substrate, deoxyadenosine triphosphate (Muller et al., 1977b); (b) inhibition of virus-induced ribonucleotide reductase by either vidarabine triphosphate or vidarabine diphosphate, which reduces the deoxyadenosine triphosphate pool and ultimately inhibits DNA synthesis (Cohen, 1972; Langelier and Buttin, 1981); (c) selective incorporation of vidarabine monophosphate into viral DNA causing a decrease in the rate of primer elongation and chain termination. This latter mechanism was suggested by several investigators (Muller et al., 1977a), but others observed random incorporation of vidarabine monophosphate into internal linkages of both viral and cellular DNAs (Pelling et al., 1981). We have shown that the efficacy of vidarabine is modified by mutation of the viral DNA polymerase, and this indicates that vidarabine is acting directly on viral DNA polymerase, resulting in inhibition of VZV replication (Kamiyama et al., 2001).

In the present study, we characterized the differences in the anti-VZV activities of acyclovir and vidarabine treatment in vitro. Intermittent treatment with acyclovir gave a 7.9 times higher IC_{50} than continuous treatment, while intermittent treatment with vidarabine gave an IC_{50} similar to continuous treatment, indicating that the anti-viral state induced by vidarabine continued for a longer time than that induced by acyclovir. Although the half-life of phosphorylated vidarabine is not known in VZV-infected cells, it is known in HSV-1-infected cells. It is reported that the half-life of ara-ATP in uninfected cells was 3.2 h compared to 9.3 h in virus-

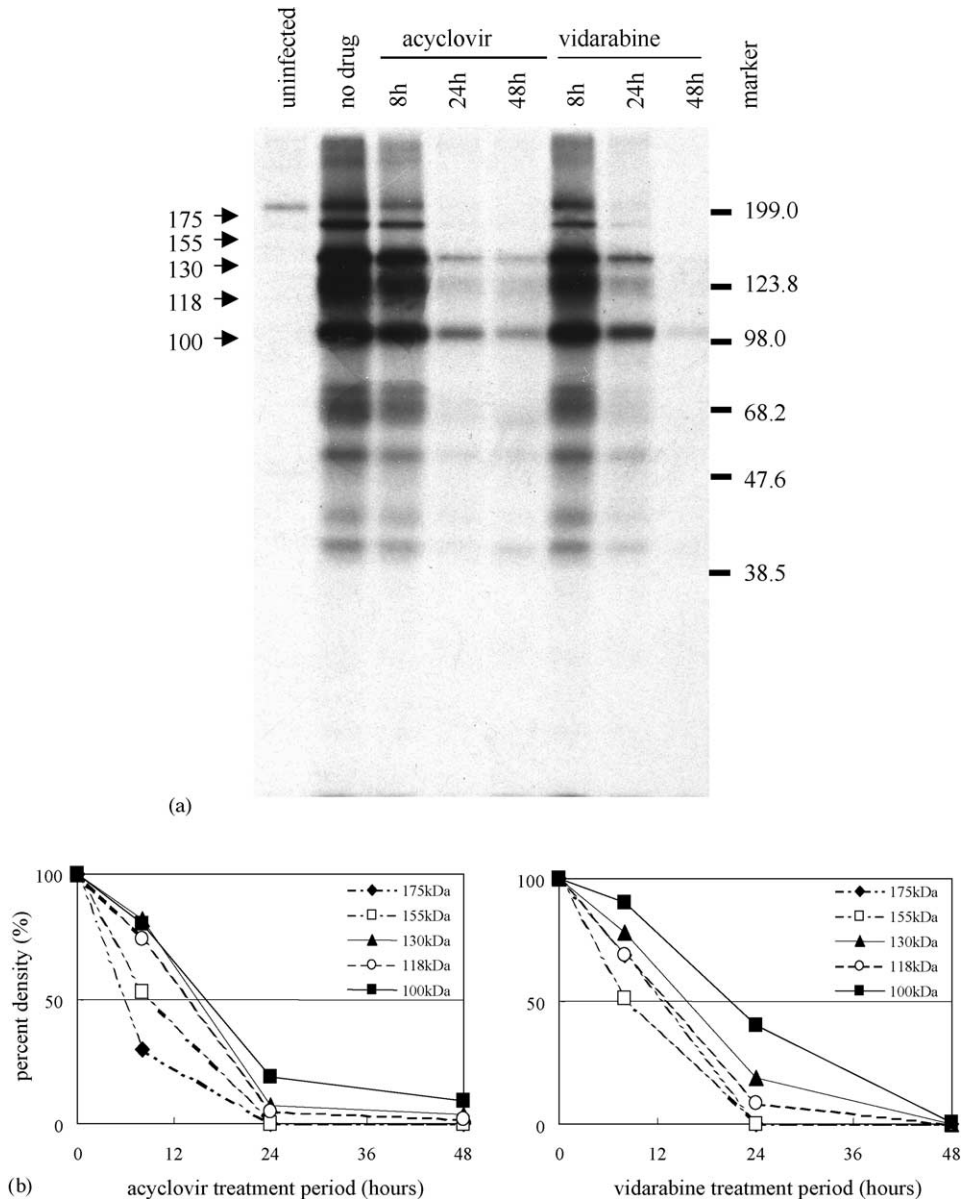

Fig. 3. Effects of acyclovir and vidarabine on VZV protein synthesis with various treatment times: (a) VZV-infected cells were treated with acyclovir or vidarabine for 8, 24, or 48 h as described in the text. Molecular weight markers used were myosin, 199 k; β-galactosidase, 123.8 k; bovine serum albumin, 98 k; glutamate dehydrogenase, 68.2 k; ovalbumin, 47.6 k; carbonic anhydrase, 38.5 k; myoglobin, 28.9 k. (b) Quantitative analysis of the intensities of VZV protein bands. Percent intensity of the bands was determined from the intensities of the bands in the absence of acyclovir and vidarabine as a standard.

infected cells (Schwartz et al., 1984). Both these times are considerably longer than the 0.8 h for phosphorylated acyclovir in VZV-infected cells and could explain the differences between vidarabine and acyclovir noted in our study (Vere Hodge and Cheng, 1993). Therefore, it is suggested that continuous treatment with acyclovir is needed to exhibit full anti-VZV activity, while vidarabine treatment once a day is equivalent to continuous treatment with acyclovir or vidarabine. The time-dependent anti-viral activity of vidarabine was similar to that of acyclovir in the plaque-reduction assay. However, only acyclovir showed a large difference in IC_{50} between the 24- and 48-h treatment periods. The IC_{50} of intermittent treatment with acyclovir corresponded to 36 h of treatment (Fig. 2), and this was the accumulated period of 8 h treatment period for 4 days. These results suggested that acyclovir needs to be present continuously for more than 48 h to exert full anti-VZV activity.

Immunoprecipitation showed that inhibitory patterns of the viral proteins by vidarabine were almost equal to that by acyclovir. This result suggested that the mechanism of vidarabine is almost the same as that of acyclovir, which means inhibition of viral DNA polymerase and consequently cessation of viral DNA synthesis.

In this study, vidarabine showed anti-VZV activity with a limited treatment period in 1 day similar to continuous treatment with acyclovir, supporting the current treatment regi-

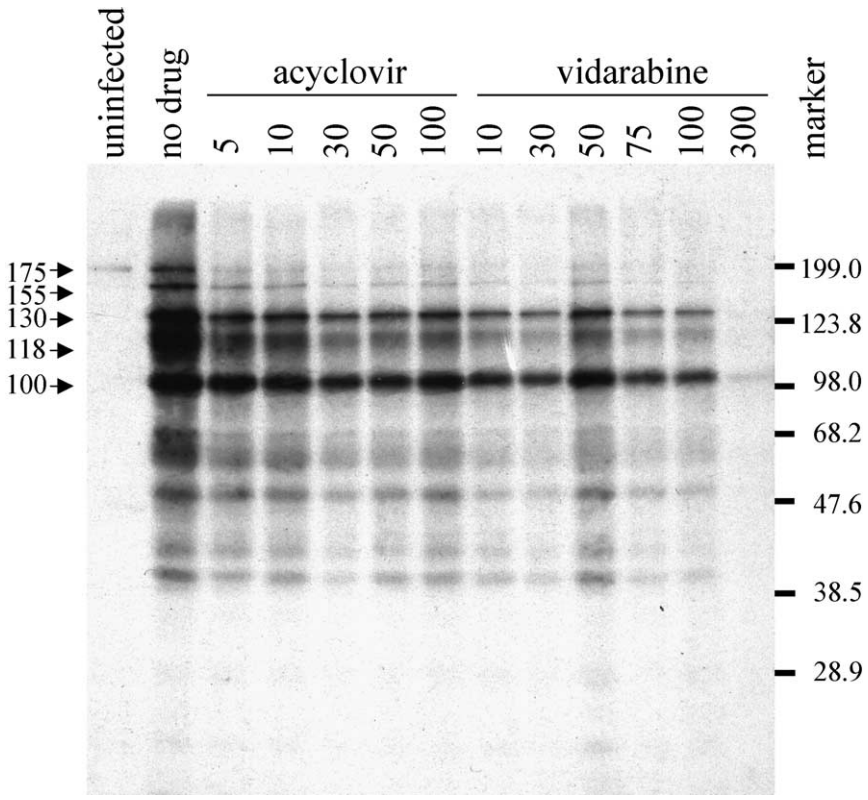

Fig. 4. Effects of acyclovir and vidarabine on VZV protein synthesis at various concentrations. VZV-infected cells were treated for 24 h with acyclovir at concentrations of 5, 10, 30, 50 and 100 μg/ml or vidarabine at concentrations of 10, 30, 50, 75, 100 and 300 μg/ml. Molecular weight markers used were myosin, 199 k; β-galactosidase, 123.8 k; bovine serum albumin, 98 k; glutamate dehydrogenase, 68.2 k; ovalbumin, 47.6 k; carbonic anhydrase, 38.5 k; myoglobin, 28.9 k.

mens of acyclovir and vidarabine. A previous study suggested that acyclovir and vidarabine were similarly active against VZV infection but that acyclovir had fewer adverse side effects (Whitley et al., 1991). However, acyclovir-resistant mutants are sensitive to vidarabine (Shiraki et al., 1990; Kamiyama et al., 2001) and acyclovir-resistant VZV infections have been successfully treated with vidarabine (Reusser et al., 1996). We have shown comparable anti-VZV activity for vidarabine and acyclovir and a longer duration of vidarabine activity. This supports the hypothesis that the current clinical protocol of once-a-day vidarabine treatment may exhibit its full anti-VZV activity for the treatment of VZV infection. Most clinicians would favor the use of foscarnet or cidofovir in the therapy of VZV-resistant viruses because vidarabine is much more toxic and is no longer available as the intravenous form in most parts of the world. However, vidarabine can still be considered as a useful and effective drug for the treatment of VZV infections.

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