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# Effect of Intensive Acyclovir Therapy during Artificial Reactivation of Latent Herpes Simplex Virus[1] (41563)

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*Abstract.* The chemotherapeutic effect of intensive acyclovir (ACV) treatment was evaluated in New Zealand white rabbits with confirmed latent herpes simplex virus type 1 (HSV-1). Acyclovir was administered orally (1 mg/ml of drinking water), topically (three times per day), and intramuscularly (16.5 mg/kg body wt twice per day) beginning 36 days after ocular inoculation and continued for 4 consecutive weeks, during which time the rabbits underwent concurrent stimulation by bilateral iontophoresis of 0.01% epinephrine into the cornea to induce reactivation of latent virus. None of the eight rabbits receiving intensive ACV shed virus in their tear film sample during 4 weeks of concurrent epinephrine iontophoresis and antiviral therapy. All five rabbits receiving placebo treatment shed HSV in tear film samples. The total number of positive ocular samples was 14 during the first week of dual treatment (5/5 animals positive) and decreased to 2 positive samples (2/5 animals) during the fourth week of dual treatment. Upon sacrifice, HSV was isolated from 8 of 16 trigeminal ganglia of ACV-treated animals and 6 of 10 placebo-treated animals. No increased resistance to ACV was detectable in any of the virus isolated from trigeminal ganglia. Antibody and cellular immune responses were not significantly different between the ACV-treated and placebo-treated groups.

Following primary infection with herpes simplex virus (HSV), a long term (probably lifelong) association appears to exist between the virus and its host. Such latent virus has been reported to reside within ganglionic neurons of both the sensory and autonomic nervous systems (1). The majority of herpetic diseases are due to reactivation of latent HSV. At present there is no known way of preventing a natural HSV infection from establishing latency or of preventing its reactivation (2). The mechanism of latency has not yet been elucidated; various hypotheses proposed to explain the phenomenon have been reviewed (3). Two important aspects of the problem were addressed by Roizman (4) in his "dynamic" and "static" state hypothesis. The "dynamic state" hypothesis suggests that the virus exists in an infectious form, perhaps slowly replicating in the host, although the immune system and, possibly, interferon limit virus spread. The "static state" hypothesis proposes that the viral genome exists in a nonreplicating form within the host cell genome as either an integrated or extrachromosomal entity.

We and others have attempted, but failed, to eradicate latent HSV infection by chronic systemic antiviral therapy (5, 6). A new approach was necessary because latent virus was not eliminated by previous treatment regimens. Therefore, we intentionally induced HSV reactivation in the nervous system by ocular epinephrine iontophoresis, during chemotherapy. Our purpose was to stimulate latently infected cells to produce virus and viral thymidine kinase while dosing the animal heavily with topical and systemic antiviral drug. The newly synthesized viral thymidine kinase in turn would activate the antiviral drug and thus inactivate the once occult virus.

We selected acyclovir (ACV) 9-(2-hydroxyethoxymethyl)guanine or acycloguanosine (ACG) because of its potent, relatively nontoxic, and specific antiviral activity against HSV (7). This antiviral activity is dependent upon its interaction with virus-specific thymidine kinase (TK) for phosphorylation to ACV-monophosphate (8). ACV-monophosphate is then further phosphorylated to di- and triphosphate forms by host cell enzymes

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(9). It is ultimately this ACV-triphosphate that interferes with viral DNA polymerase activity and serves as a terminator for growing viral DNA molecules (10).

**Materials and Methods.** *Animals and virus inoculation.* Young adult male New Zealand white rabbits, weighing approximately 3 kg each, were inoculated with $10^6$ plaque-forming units (pfu) of HSV (McKrae) per eye without scarification. Primary ocular infection was confirmed by slit lamp biomicroscopy and by virus isolation from tear film on Day five after inoculation. Rabbits surviving the acute HSV infection for 28 days were considered latently infected.

*Chemotherapy ACV.* (Kindly provided by Burroughs Wellcome Co, Research Triangle Park, N.C.) was administered to latently infected animals beginning 36 days after inoculation of virus. Starting 12 hr before the epinephrine iontophoresis procedure, the drug was given as follows: An oral dose of 6–7 mg/kg/day of ACV in the drinking water (approximately 200 ml of water, 1 mg/ml, was displaced daily by each rabbit and estimating that one-tenth of the water was actually consumed or absorbed); intramuscular (im) dose of 16.5 mg/kg twice a day; and a topical dose of 3% Zovirax ophthalmic ointment in both eyes three times each day. Intensive ACV therapy was carried out for 4 consecutive weeks (days 36–63 postinoculation), during which time concurrent ocular stimulation by epinephrine iontophoresis was used in an attempt to induce reactivation of CNS latent HSV.

*Reactivation of latent HSV.* Attempts were made to induce reactivation of latent HSV in rabbits between 36–63 days postinoculation. The method employed has been described recently by Kwon *et al.* (11). Briefly, anesthetized rabbits were fitted with an eye cup containing 0.01% epinephrine. The anode (+) terminal was placed in the epinephrine solution and the cathode (−) terminal was attached to the ear. A direct current (0.6 mA) was applied for 6–8 min. This procedure was performed on each eye once a day for 3 consecutive days and was repeated on a weekly basis for 4 weeks without apparent adverse effect.

*Isolation of virus from tear film.* Both eyes of each rabbit were cultured for virus daily throughout the study. The cornea and lower cul de sac of each eye was carefully washed with 0.5 ml physiological saline and collected with a sterile pipet. Infectious HSV in the eye was quantitated by the plaque assay method in rabbit kidney cells (RK), as previously described (12).

*Isolation of latent virus from trigeminal ganglion.* On Day 84 postinoculation (3 weeks following termination of chemotherapy), rabbits were sacrificed by iv injection of sodium pentobarbital. Tissues were removed aseptically, minced, enzymatically dispersed, and cocultivated with RK cells to enhance the recovery of latent virus from neuronal tissues, as previously described (13).

*Determination of ED-50 values.* ED-50 values, i.e., the concentration of drug giving 50% plaque reduction, were determined on HSV isolated from trigeminal ganglia of rabbits treated intensively with ACV. Confluent monolayers of RK cells in 60-mm petri dishes were inoculated with approximately 100 pfu of the isolated HSV and adsorbed at room temperature for 1 hr. The inoculum was removed and replaced with methyl cellulose overlay medium with or without threefold serial dilutions of ACV. The monolayers were fixed after 72 hr, plaques counted, and the average numbers of plaques formed in the presence of each drug concentration were determined. The ED-50 value was then calculated for each isolate.

*Immunological assays.* Peripheral blood was collected from the central artery of the ear using heparinized Vacutainer tubes. Buffy-coat lymphocytes and blood plasma were collected after centrifugation (800*g*). Lymphocytes were purified using Ficoll isopaque (Pharmacia) gradient centrifugation (14). Plasma was collected and stored at −20°C. Lymphocyte stimulation assays using purified peripheral blood lymphocytes were done using a modification of the procedure of Meyers-Elliott and Chitjian (15). Briefly, lymphocytes ($1 \times 10^6$ cells/ml) were incubated in microtiter plates (Falcon) at 35°C in a humidified (5% $CO_2$:95% air) incubator for 96 hr. Cells were incubated with media, mitogens (PHA-P, 250 μg/ml, or anti-rabbit IgG, 250 μg/ml) or antigens (uv-irradiated culture fluid from HSV-infected cells or uv-irradiated culture fluid from mock infected cells). At 24 hr prior to termination of culture, 1 μCi of $^3$H-thy-

midine (New England Nuclear) was added to each well. Cultures were harvested 24 hr later with a MASH (MA Bioproducts) and processed for liquid scintillation counting. The stimulatory index (SI) was computed as follows:

$$SI = \frac{\text{cpm from lymphocyte cultures with antigen of mitogen}}{\text{cpm from lymphocyte cultures with media}}$$

Serum neutralization (SN) titers were determined by microtitration techniques (16). Twofold dilutions of serum prepared in tissue culture media were incubated with $1 \times 10^3$ pfu of HSV for 30 min. Triplicate samples (100 μl) of each dilution were then cultured with RK cells. Cells were incubated at 35°C for 5 days. Titer was expressed as the reciprocal of the highest serum dilution showing no cytopathic effect (CPE) in more than 50% of the wells at termination of incubation.

The solid-phase enzyme-linked immunosorbant assay (ELISA) technique was performed using a modification of the procedure of Voller *et al.* (17). Briefly, HSV antigens (30 μg/ml) in 0.1 *M* carbonate buffer (pH 9.6) were added to polyvinyl plates (Falcon) and incubated at 4°C for 18 hr. The HSV antigen was prepared from supernatant fluid from infected cell cultures by a low-speed spin to pellet virions and a second high-speed spin in 0.1 *M* phosphate-buffer wash. Plates were washed four times with BGPT buffer (1% bovine serum albumin, 0.3% gelatin, 0.2% tween 80, phosphate-buffer solution, pH 7.2). Following a 30 min incubation with BGP buffer (BGPT without Tween 80) and subsequent washing four times with BGPT, dilutions of the rabbit sera (100 μl/well) made in BGP buffer were allowed to incubate for 1 hr at 35°C. After plates were washed four times with BGPT buffer, 100 μl of *Staphylococcus* protein A-HRP (Zymed) diluted 1:3000 was added to each well and incubated for 30 min at 35°C. Following washing an additional four times with BGP buffer, the presence of protein A-HRP was detected using an enzyme substrate composed of 0.04% *o*-phenylenediamine (Sigma), 0.01% hydrogen peroxidase, and 0.05 *M* citrate buffer (pH 5.0). The plates containing enzyme substrate were allowed to incubate at ambient temperature for 30 min in a dark box. The optical density (OD) was determined using the Micro ELISA Reader (Dynatech). The titer was the reciprocal of the highest serum dilution giving an OD equal to two times that of the (background = 0.10).

TABLE I. INDUCTION OF HSV-1 INTO TEAR FILM BY BILATERAL EPINEPHRINE IONTOPHORESIS BEFORE CHEMOTHERAPY[a]

| Rabbit No. | Eye | Day of epinephrine iontophoresis cycle 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | OS | ● | | | | | | |
| | OD | | | | | | | |
| 2 | OS | | | | | | | |
| | OD | | | | | | ● | |
| 3 | OS | | | | | ● | ● | ● |
| | OD | | | | ● | ● | ● | ● |
| 4 | OS | | | | | | | |
| | OD | | | | ● | | | |
| 5 | OS | | | | | | | |
| | OD | ● | | | | | | |
| 6 | OS | | | | ● | | | |
| | OD | | | ● | | | | |
| 7 | OS | | | | ● | ● | ● | |
| | OD | | | | | | | |
| 8 | OS | | | | ● | | | |
| | OD | | | ● | | | | |
| 9 | OS | | | | ● | | | |
| | OD | | | | | | | |
| 10 | OS | | | | ● | | | |
| | OD | | | | | | | |
| 11 | OS | | | | | | | |
| | OD | | | | ● | | | |
| 12 | OS | | | ● | ● | | | |
| | OD | | | | | | | |
| 13 | OS | | | | | | ● | ● |
| | OD | | | | | | | |

[a] Epinephrine iontophoresis was carried out on Days 1–3; tear film samples from both eyes were collected daily (Days 1–7) and examined for HSV by inoculation on RK cells. Filled circles indicate HSV-positive tear film samples.

**Results.** *Reactivation of Latent HSV.* Twenty-eight days following ocular inoculation with HSV-1, rabbits were stimulated by iontophoresis with 0.01% epinephrine to induce reactivation of latent virus. Thirteen rabbits had positive HSV tear film samples within 7 days of initiation of the stimulation procedure (Table I), which was considered a confirmation of latent HSV infection. Most virus-positive ocular cultures were taken between Day 3 and 6 of the first cycle of epinephrine iontophoresis. One rabbit had seven

TABLE II. HSV ISOLATION DURING CONCURRENT INTENSIVE ACV THERAPY AND STIMULATION TO INDUCE LATENT HSV[a]

| Rabbit No.[b] | Treatment | Cycle days PI | Number of positive ocular samples during weekly cycle of concurrent therapy and stimulation | | | |
|---|---|---|---|---|---|---|
| | | | 1 (36–42) | 2 (43–49) | 3 (50–56) | 4 (57–63) |
| 1 | ACV | | 0 | 0 | 0 | 0 |
| 2 | ACV | | 0 | 0 | 0 | 0 |
| 8 | ACV | | 0 | 0 | 0 | 0 |
| 9 | ACV | | 0 | 0 | 0 | 0 |
| 10 | ACV | | 0 | 0 | 0 | 0 |
| 11 | ACV | | 0 | 0 | 0 | 0 |
| 12 | ACV | | 0 | 0 | 0 | 0 |
| 13 | ACV | | 0 | 0 | 0 | 0 |
| Totals | | | 0 | 0 | 0 | 0 |
| 3 | Placebo | | 5 | 1 | 0 | 0 |
| 4 | Placebo | | 4 | 0 | 1 | 1 |
| 5 | Placebo | | 2 | 1 | 2 | 0 |
| 6 | Placebo | | 2 | 1 | 0 | 0 |
| 7 | Placebo | | 1 | 0 | 1 | 1 |
| Totals | | | 14 | 3 | 4 | 2 |

[a] Bilateral epinephrine iontophoresis was carried out on Days 1–3 during each of four cycles; tear film samples from both eyes were collected daily and virus isolation attempted in RK.

[b] Rabbit numbers correspond to those in Table I.

positive ocular cultures during this 4-day period.

*HSV shedding during concurrent therapy and stimulation.* The 13 rabbits with confirmed latent HSV infection were divided into two treatment groups (Table II). The intensive ACV treatment regimen was topical, parenteral, and oral as outlined above. The placebo group (five rabbits) received topical ointment (same petrolatum base without drug), in saline, and water without drug. Concurrent with this therapy, all rabbits were subjected to weekly 3-day cycles of epinephrine iontophoresis to induce reactivation of latent HSV. This dual regimen of chemotherapy and stimulation was performed for four consecutive weekly cycles.

HSV was not detected in tear film samples taken from the eight rabbits receiving intensive ACV therapy and epinephrine iontophoresis. However, HSV was isolated from tear film samples of all five rabbits receiving placebo therapy. The total number of positive ocular samples was 14 during cycle 1 (5/5 animals positive). During cycles 2 and 3, the total number of positive ocular samples decreased to 3 and 4, respectively (3/5 animals positive). After cycle 4, only two rabbits shed HSV in one eye, each on a single occasion.

*HSV shedding following therapy.* Virus isolation data beginning after termination of therapy is presented in Table III. A period was allowed to elapse between termination of concurrent therapy and stimulation (Day 63) and the final attempt to induce reactivation of latent HSV commencing on Day 77. The purpose of this time interval and stimulation were to permit any virus still present in the drug-treated animals to undergo replication and reach a level that would be more easily detected (i.e. an amplification period). Five of the 8 (62.5%) ACV-treated rabbits shed virus one time during this final stimulation attempt. The five rabbits with HSV-positive tear film samples had the same eye involved after therapy as before therapy. Of the three rabbits which had virus negative tear film cultures, two had virus isolated from their cocultivated trigeminal ganglia.

"Latent" HSV was isolated from 8 of 16 trigeminal ganglia of ACV-treated animals and 5 of 10 trigeminal ganglia of placebo-treated animals (Table IV). Ability to isolate HSV from the trigeminal ganglia did not always

TABLE III. INDUCTION OF HSV-1 INTO TEAR FILM BY BILATERAL EPINEPHRINE IONTOPHORESIS 14 DAYS FOLLOWING COMPLETION OF CONCURRENT THERAPY AND STIMULATION

| | | | Day of epinephrine iontophoresis cycle | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Rabbit No. | Treatment | Eye | 1[a] | 2[a] | 3[a] | 4 | 5 | 6 | 7 |
| 1 | ACV | OS | | | | | | | |
| | | OD | | | | | | | |
| 2 | ACV | OS | | | | | | | |
| | | OD | | | | | | ● | |
| 8 | ACV | OS | | | ● | ● | | | |
| | | OD | | | | | | | |
| 9 | ACV | OS | | | | | | | |
| | | OD | | | | | | | |
| 10 | ACV | OS | | | | ● | | | |
| | | OD | | | | | | | |
| 11 | ACV | OS | | | | | | | |
| | | OD | | | | | | | |
| 12 | ACV | OS | | | ● | | | | |
| | | OD | | | | | | | |
| 13 | ACV | OS | | ● | | | | | |
| | | OD | | | | | | | |
| 3 | Placebo | OS | | | | | ● | | |
| | | OD | | | | | ● | | |
| 4 | Placebo | OS | | | | ● | | | |
| | | OD | | | | ● | | | |
| 5 | Placebo | OS | | | | | | | |
| | | OD | | | | | ● | ● | ● |
| 6 | Placebo | OS | | | | | | | |
| | | OD | | | | | | | |
| 7 | Placebo | OS | | | | | | | |
| | | OD | | | | | | | |

[a] Bilateral epinephrine iontophoresis was carried out on Days 1–3 and tear film samples from both eyes were collected daily (Days 1–7) and examined for HSV by inoculating RK cells, filled circles (●) indicate HSV positive tear film samples.

coincide with the earlier appearance of virus in the tear film samples following epinephrine iontophoresis. For example, when one rabbit (No. 3) was initially stimulated, the left eye shed virus three times, yet no virus was isolated from the left trigeminal ganglia. When another rabbit (No. 13) was first stimulated, the left eye shed virus twice, yet no virus was isolated from the left trigeminal ganglia. It is not known why virus could not be routinely isolated from the ipsilateral trigeminal ganglia known to be shedding virus.

ED-50 values for a single HSV isolate taken from four of five ACV-treated rabbits with positive trigeminal ganglia are shown in Table IV. The ED-50 for the original HSV-1 (McKrae) used to establish the latent infection was 0.1 $\mu M$ and was 0.1–0.2 $\mu M$ for the trigeminal ganglia isolates. Thus, no increased

TABLE IV. RECOVERY OF HSV-1 ISOLATES FROM TRIGEMINAL GANGLIA FOLLOWING INTENSIVE ACV THERAPY AND DETERMINATION OF THEIR ED-50 VALUES

| Rabbit No. | Treatment | HSV positive (right) | Trigeminal ganglion (left) | ED-50 ($\mu M$) |
|---|---|---|---|---|
| 1 | ACV | | | — |
| 2 | ACV | | | — |
| 8 | ACV | ● | ● | 0.1 |
| 9 | ACV | ● | ● | 0.1 |
| 10 | ACV | ● | | 0.2 |
| 11 | ACV | ● | ● | 0.1 |
| 12 | ACV | ● | | ND |
| 13 | ACV | | | — |
| 3 | Placebo | ● | | ND |
| 4 | Placebo | ● | ● | 0.1 |
| 5 | Placebo | | ● | 0.1 |
| 6 | Placebo | | ● | ND |
| 7 | Placebo | ● | | ND |

TABLE V. SERUM ANTIBODY LEVEL DURING CONCURRENT STIMULATION AND INTENSIVE ACYCLOVIR CHEMOTHERAPY

| Group | Number of animals | Average antibody titer[a] | |
|---|---|---|---|
| | | Serum neutralization (number samples)[b] | ELISA (number samples)[c] |
| ACV | 5 | 51 (23)<br>Range: 32–128 | 4492 (26)<br>Range: 1600–6400 |
| Control | 4 | 48 (14)<br>Range: 32–64 | 4100 (16)<br>Range: 1600–6400 |

[a] Peripheral blood taken on a weekly basis 1 day after the 3-day cycle of iontophoresis was stopped (blood not obtained from each animal on each week).

[b] Titer = reciprocal of highest dilution inhibiting CPE in 50% monolayers using $1 \times 10^3$ PFU of HSV-1 per monolayer.

[c] Titer = reciprocal of highest dilution giving an OD = 2 × background using protein A-HRP conjugate.

resistance to ACV was evident with these isolates.

*Immune responses during acyclovir therapy.* As shown in Table V, the serum neutralization titers and ELISA titers showed no significant difference between the ACV-treated and the control groups. There was no greater than a twofold dilution difference in anti-HSV serum titers between the six bleedings collected on a weekly basis from the same animal during the treatment period. In all instances, the ELISA titers were approximately 100-fold higher than SN titers for the same sera.

The cellular immune responses studied (Table VI) also showed no significant difference between the ACV-treated and control groups. The positive lymphocyte responses to mitogens and antigens were equivalent in both groups. We elected to document positive responses rather than average SI because of variability between weekly assays.

**Discussion.** Eradication of HSV from the nervous system has proven to be a difficult task. The new approach reported here was to determine whether concurrent therapy and artificial reactivation could rid the nervous system of latent HSV.

As reported previously by Kwon *et al.* (11), we confirm in the present study that iontophoresis of 0.01% epinephrine to the cornea of latently infected rabbits reproducibly induces HSV reactivation with virus shedding

TABLE VI. LYMPHOCYTE RESPONSE DURING CONCURRENT STIMULATION AND INTENSIVE ACYCLOVIR CHEMOTHERAPY

| Group | Number of animals[b] | Percent of cultures with positive response[a] | | |
|---|---|---|---|---|
| | | HSV antigen (mixed)[c] | PHA-P (T-cells)[d] | Anti-rabbit IgG (B-cells)[d] |
| ACV | 5 | 35 (7/20) | 94 (16/17) | 76 (13/17) |
| Control | 4 | 33 (6/18) | 94 (15/16) | 88 (14/16) |

[a] Positive response is defined by the stimulatory index (SI) of peripheral blood lymphocyte cultures:

$$SI = \frac{\text{cpm from lymphocyte cultures with antigen or mitogen}}{\text{cpm from lymphocyte cultures with media}}$$

[b] Cultures of lymphocyte from peripheral blood were established on a weekly basis 1 day after the 3-day cycle of iontophoresis.

[c] A positive response is a SI $\geq$ 1.5 determined by subtracting the SI of cultures with mock-infected cell antigen from the SI of cultures with HSV-infected cell antigen.

[d] A positive response is a SI $\geq$ 3.0.

into the tear film. This procedure provides a dependable way to initiate reactivation of latent virus, and hopefully insure that HSV TK enzyme is available to activate the ACV. Even though it has been reported that some HSV TK activity was present in ganglia of latently infected animals (18, 19) and that limited transcription of the HSV genome did occur during latency (20), we felt that ACV therapy would have greater opportunity to be successful if the latent virus could be triggered to express itself during therapy. ACV was administered by multiple routes in the hope that effective antiviral levels could be achieved and maintained.

Topical treatment was purposely employed to prevent or minimize reinfection of trigeminal ganglia following virus replication on the cornea surface, lessening centripetal spread of virus to neurons from peripheral tissues. If ganglionic reinfection from peripheral tissues is necessary to maintain the latency (i.e., "round-trip" hypothesis), then this mechanism of maintaining the latent state should be interrupted by topical treatment (21).

Even though our studies show that ACV did not eradicate latent HSV infection, the drug appeared to yield beneficial results by reducing the incidence of inducible recurrences during chemotherapy (Table II). It may be that extending the period of dual treatment or modifying the drug or treatment regimen would enhance further the desired affect.

Two important observations regarding virus isolation are apparent from this study. First, even without chemotherapy the frequency of inducible recurrences of latent virus in the tear film appears to decrease with time. This is consistent with the findings of other investigators that the appearance of virus and viral antigen in latency decreases with time in both mice (22) and rabbits (11, 23). The possibility thus exists that latent HSV could be eradicated from the nervous system solely by repeated and controlled reactivations. The other observation is that intensive ACV therapy appears to reduce the incidence of HSV shedding during the time of concurrent therapy and stimulation. However, antiviral activity resulting from high levels of ACV in tear films due to intensive drug therapy could account for failure to detect tear film virus following stimulation. But, tear film samples were collected early each morning, before the daily drug treatments were administered. Furthermore, when known amounts of HSV were mixed with tear film samples from ACV- and placebo-treated eyes, no difference in antiviral activity was observed. This would suggest that our inability to isolate virus during ACV therapy was not due to the presence of residual ACV in the tear film sample.

ACV-resistant strains of HSV have been readily inducible *in vitro*. This resistance has been associated with either the TK locus or the DNA polymerase locus (24, 25). HSV resistance to ACV in humans has recently been reported (26, 27). The extent of *in vivo* ACV resistance is not yet known because of the relatively short period of time that ACV has been available. In our study, HSV isolates from animals having undergone 4 weeks of intensive ACV treatment showed no resistance in the isolates tested. This preliminary finding appears to support the "static" hypothesis because, if latent virus were undergoing continuous replication in the presence of this antiviral drug, there would be a selection process favoring drug resistant strains. If the "round-trip" hypothesis, which states that latency is maintained by reseeding of neurons by virus produced in peripheral tissues, were valid, one might expect HSV isolated from neural tissue following intensive ACV therapy to display increased drug resistance. This was not the case.

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