Assessment of a Selective Inhibitor of Herpes Simplex Virus Thymidine Kinase (L-653,180) as Therapy For Experimental Recurrent Genital Herpes

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Herpes simplex virus (HSV)-coded thymidine kinase (TK) is important in efficient reactivation of latent infection. These studies were designed to investigate whether treatment of latently infected animals with a TK inhibitor altered the natural history of recurrent HSV disease. 9-[[*(Z)*-2-(hydroxymethyl)cyclohexyl]methyl]guanine (L-653,180) is a potent and selective nonsubstrate inhibitor of HSV TK which can suppress or delay reactivation of HSV-1 from latently infected cells *in vitro* without affecting viral replication. In an initial study, six female Hartley guinea pigs were treated with L-653,180 in their diet (25 mg/30 g of food) and water (300 mg/liter) for 7 days. Blood, urine, kidney, liver, spinal cord, and cerebral cortex specimens were collected. L-653,180 was detected in all specimens at concentrations which, although low, were higher than the *in vitro* 50% inhibitory concentration of the drug against HSV TK. In the second study, 20 female Hartley guinea pigs were randomized into two groups following recovery from primary genital HSV-2 infection. One group received L-653,180 in diet and water for 4 weeks beginning 21 days postinoculation. Animals were examined daily for recurrent lesions for 10 weeks. Treated animals experienced fewer recurrences during the treatment period but the results were not significantly different from results with controls. During the first 2-week posttreatment period, L-653,180-treated animals had significantly fewer recurrences than control animals ($P = 0.02$). Over the entire 10-week observation period, treated animals experienced fewer recurrences ($P = 0.06$). These results suggest that inhibitors of viral TK may be useful in limiting reactivation of latent virus and thus recurrent infections. In these experiments, the amount of drug that could be administered to the animals was limited by its poor solubility. Further studies with more potent and soluble inhibitors of HSV TK appear to be warranted.

Recurrent herpes simplex virus (HSV) infections result from the reactivation of latent virus present in sensory ganglia (9). The current therapeutic approach for control of recurrent HSV disease involves the use of antiviral agents that prevent the replication of the reactivated virus (6). An alternative strategy for the control of herpetic recurrences would be to interfere with the reactivation process. One possible target for such a strategy might be the virus-encoded enzyme thymidine kinase (TK). HSV TK is required for the efficient replication of virus in nondividing cells (4). TK-deficient (TK⁻) mutants replicate well at the portal of entry (e.g., vaginal epithelium) but replicate poorly in nondividing neuronal cells (i.e., the site of latent infection) (18, 20). TK⁻ HSV-1 or HSV-2 can establish latent infection; however, recovery of latent TK⁻ virus by explant cocultivation is difficult, suggesting that TK may be important in the efficient reactivation of latent virus (3, 11, 19). This hypothesis is further supported by the observation that guinea pigs infected with TK⁻ HSV-2 experience infrequent recurrent episodes (15). Thus, it is possible that selective inhibition of HSV TK in latently infected hosts may provide a novel strategy for the control of recurrent HSV disease.

The compound 9-[[*(Z)*-2-(hydroxymethyl)cyclohexyl]methyl]guanine (L-653,180) is a potent and selective nonsubstrate inhibitor of HSV TK (1). It has no antiviral activity in tissue culture, but it prevents HSV-1 reactivation from latently

infected human embryo lung cells (14) and delays reactivation of HSV-1 from latently infected mouse trigeminal ganglia (1). In this study, we used the guinea pig model of recurrent genital HSV-2 infection to test the hypothesis that treatment of latently infected animals with an inhibitor of viral TK would reduce herpetic recurrences. We report that L-653,180 could be detected in the tissues of treated animals at concentrations greater than the *in vitro* 50% inhibitory concentration (IC₅₀) for HSV TK and that treated animals did show evidence of reduced recurrent HSV-2 disease. Details of the chemical synthesis of L-653,180 are also presented.

MATERIALS AND METHODS

Synthetic methods. The synthesis of 9-[[*(Z)*-2-(hydroxymethyl)cyclohexyl]methyl]guanine is schematically represented in Fig. 1.

(*Z*)-2-(Benzoyloxymethyl)cyclohexanemethanol (compound II). To a solution of 50.0 g (0.346 mol) of *cis*-1,2-cyclohexanediol (compound I) and 35 ml of pyridine in 400 ml of methylene chloride stirred under nitrogen at 0°C was added dropwise 40.25 ml (48.7 g; 0.346 mol) of benzoyl chloride. The mixture was stirred overnight at room temperature, concentrated *in vacuo*, and partitioned between ethyl acetate and water. The residue from concentration of the organic layer was chromatographed on silica gel (hexane-ethyl acetate) to give 43.9 g (51%) of product as an oil, homogeneous by thin-layer chromatography (TLC) (4:1 hex-

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FIG. 1. Synthesis of 9-[(Z)-2-(hydroxymethyl)cyclohexyl]methylguanine. *i*, BzCl-Cl-CH₂-N-CH₂-Cl₂; *ii*, CBr₄-Ph₃P-CH₂-Cl₂; *iii*, 2-amino-6-benzoyloxypurine/NaH/DMF (60 to 65°C, 19 h); *iv*, trifluoroacetic acid; *v*, NaOMe/MeOH.

ane-ethyl acetate). 200 MHz ¹H nuclear magnetic resonance (NMR) (CDCl₃): δ 1.2 to 1.8 (complex m, 8H), 1.96 (m, 1H), 2.26 (m, 1H), 3.67 (m, 2H), 4.35 (m, 2H), 7.35 to 7.6 (complex m, 3H), 8.02 (d, *J* = 8 Hz, 2H).

[(Z)-2-(Benzoyloxymethyl)cyclohexyl]methyl bromide (compound III). A solution of 43.0 g (0.173 mol) of compound II and 86.2 g (0.26 mol) of carbon tetrabromide in 400 ml of dry methylene chloride was stirred at room temperature as 54.3 g (0.207 mol) of triphenylphosphine in 100 ml of methylene chloride was added dropwise over 2 h. After 4 days of stirring at room temperature, concentration of the mixture gave a dark residue, which was triturated with 1:1 hexane-ethyl acetate. The insoluble solid was removed by filtration, and the residue was chromatographed on silica gel (hexane-ethyl acetate), yielding 43.8 g (81%) of product as an oil, homogeneous by TLC (9:1 hexane-ethyl acetate). 300 MHz ¹H NMR (CDCl₃): δ 1.35 to 1.7 (complex m, 8H), 2.16 (m, 1H), 2.40 (m, 1H), 3.47 (m, 2H), 4.32 (m, 2H), 7.44 (dd, *J* = 8 Hz, 8 Hz, 2H), 7.56 (t, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 2H). Elemental analysis: calculated for C₁₅H₁₉BrO₂: C, 57.89%; H, 6.15%; Br, 25.67%; found: C, 58.03%; H, 6.16%; Br, 25.76%.

2-Amino-9-[(Z)-2-(benzyloxymethyl)cyclohexyl]methyl-6-benzoyloxypurine (compound IV). A solution of 27.8 g (0.115 mol) of 2-amino-6-benzoyloxypurine in 275 ml of dry dimethylformamide (DMF) was stirred under N₂ and treated portionwise with 4.8 g (0.12 mol) of sodium hydride (60% in mineral oil). After 30 min, a solution of 39.4 g (0.126 mol) of [(Z)-2-(benzyloxymethyl)cyclohexyl]methyl bromide (compound III) in 30 ml of dry DMF was added dropwise. The mixture was stirred for 19 h at 60°C and then cooled, neutralized with glacial acetic acid, and concentrated. The residue was chromatographed on silica gel (elution with 0.5 to 1% methanol in methylene chloride) to isolate the desired 9-isomer (compound IV), while the 7-isomer was eluted with higher concentrations (2 to 6%) of methanol. Trituration of

the residual 9-isomer with hexane yielded 11.5 g (21%) of white solid (mp, 153 to 154°C). Similar treatment of the 7-isomer gave 8.8 g of white solid (mp, 70 to 72°C). 300 MHz ¹H NMR (CDCl₃): 9-isomer (IV) δ 1.3 to 1.7 (complex m, 8H), 2.20 (m, 1H), 2.36 (m, 1H), 4.04 (dd, *J* = 10 Hz, 14 Hz, 1H), 4.15 (dd, *J* = 5.5 Hz, 14 Hz, 1H), 4.35 (dd, *J* = 7 Hz, 11.5 Hz, 1H), 4.51 (dd, *J* = 7.5 Hz, 11.5 Hz, 1H), 4.65 (s, 2H), 5.51 (s, 2H), 7.25 to 7.6 (complex m, 9H), including 7.57 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 2H). Fully decoupled partial ¹³C NMR (CDCl₃): δ 43.6 (CH₂N), 115.6 (C⁵), 139.7 (C⁸), 154.4 (C⁴). In the gated coupled spectrum, C⁵ appeared as a simple doublet (³*J*_{CH} = 11.6 Hz) due to 3-bond coupling to H-8, while C⁴ appeared as a broad multiplet due to 3-bond coupling to side chain CH₂ and H-8. Mass spectrum (fast atom bombardment) *m/e* 472 (M + H)⁺. 300 MHz ¹H NMR (CDCl₃): 7-isomer δ 1.1 to 1.6 (complex m, 8H), 1.95 (m, 1H), 2.13 (m, 1H), 4.05 (dd, *J* = 10.5 Hz, 13.5 Hz, 1H), 4.1 to 4.3 (m, 3H), 4.83 (s, 2H), 5.43 (ABq, 2H), 7.25 to 7.6 (complex m, 8H), 7.69 (s, 1H), 7.95 (d, *J* = 8H). Fully decoupled partial ¹³C NMR (CDCl₃): δ 48.9 (CH₂N), 107.0 (C⁵), 144.9 (C⁸), 163.9 (C⁴). In the gated coupled spectrum, C⁵ appeared as a broad multiplet due to 3-bond coupling to side chain CH₂ and H-8, while C⁴ appeared as a simple doublet (³*J*_{CH} = 12.5 Hz) due to 3-bond coupling to H-8. Mass spectrum (fast atom bombardment) *m/e* 472 (M + H)⁺. Elemental analysis: calculated for C₂₇H₂₉N₅O₃: C, 68.78%; H, 6.20%; N, 14.68%; found for the 9-alkylated isomer (compound IV): C, 68.84%; H, 6.26%; N, 14.96%; calculated for C₂₇H₂₉N₅O₃ · H₂O: C, 66.32%; H, 6.45%; N, 14.32%; found for the 7-isomer: C, 65.94%; H, 6.11%; N, 14.57%.

9-[(Z)-2-(Benzoyloxymethyl)cyclohexyl]methylguanine (compound V). A solution of 10.63 g (22.5 mol) of compound IV in 100 ml of trifluoroacetic acid was stirred for 2.5 h and then evaporated in a nitrogen stream. The residual oil was reconcentrated twice from methanol and then triturated with diethyl ether to provide a white solid. Yield, 8.36 g (97%); mp, 315 to 317°C decomposed; homogeneous by TLC (9:1, chloroform-methanol). 300 MHz ¹H NMR (dimethyl sulfoxide-*d*₆): δ 1.2 to 1.7 (complex m, 8H), 2.10 (m, 1H), 2.33 (m, 1H), 4.03 (d, *J* = 8 Hz, 2H), 4.35 (m, 2H), 4.35 (m, 2H), 6.38 (br s, 2H), 7.52 (dd, *J* = 8 Hz, 8 Hz, 2H), 7.56 (t, *J* = 8 Hz, 1H), 7.73 (s, 1H), 7.96 (d, *J* = 8 Hz, 2H), 10.47 (br, 12H). Elemental analysis: calculated for C₂₀H₂₃N₅O₃: C, 62.98%; H, 6.08%; N, 18.36%; found: C, 63.03%; H, 6.07%; N, 18.58%.

9-[(Z)-2-(Hydroxymethyl)cyclohexyl]methylguanine (compound VI). A suspension of 8.01 g (21 mmol) of compound V in 750 ml of methanol was treated with 40 ml (40 mmol) of freshly prepared 1 M sodium methoxide in methanol. The mixture was stirred under nitrogen at room temperature for 6 days. The solution was neutralized with glacial acetic acid and concentrated in vacuo. Trituration of the residue with acetone gave a white solid, which was recrystallized from water-methanol to yield 5.01 g (82%); mp, 305 to 307°C decomposed; homogeneous by TLC (90:10:1, chloroform-methanol-water). 300 MHz ¹H NMR (dimethyl sulfoxide-*d*₆): δ 1.1 to 1.8 (complex m, 9H), 2.18 (m, 1H), 3.3 to 3.55 (complex m, 2H, partially obscured by H₂O peak), 3.90 (m, 2H), 4.46 (t, *J* = 5 Hz, 1H), 6.39 (br s, 2H), 7.66 (s, 1H), 10.44 (br s, 1H). UV (phosphate buffer, pH 7.0) λ_{max}: 252 nm (ε 12,600). Elemental analysis: calculated for C₁₃H₁₉N₅O₂ · 0.75 H₂O: C, 53.68%; H, 7.10%; N, 24.08%; found: C, 54.01%; H, 7.49%; N, 23.87%.

Animals. Female Hartley guinea pigs weighing 350 to 400 g were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) and housed under American Associa-

tion for Accreditation of Laboratory Animal Care (AAALAC)-approved conditions.

TK inhibition assay. The assay mixture contained, in a final volume of 100 μ l, 50 mM Tris-HCl, pH 7.5; 5 mM $MgCl_2$; 5 mM ATP; 2 mM dithiothreitol, 2.5 mM NaF; 10 μ M [methyl- ^{14}C]thymidine; 0.1 U of HSV-1 TK; and the inhibitor or blank. After 10 min of incubation at 30°C, 60 μ l of the reaction mixture was applied to a DEAE-paper disc (Whatman DE81; 2.5-cm diameter) and after 5 min the disc was placed in a dish containing ethanol. The discs were washed three times in the ethanol, dried, placed in scintillation vials with scintillation mixture, and counted.

Quantitation of L-653,180 in plasma and tissues. To determine whether animals would accept L-653,180 orally and to establish the distribution of drug in tissues following treatment, guinea pigs were randomized into two groups; one group ($n = 6$) was administered L-653,180 ad libitum in their diet (25 mg of drug/30 g of food) and drinking water (300 mg of drug/liter), while the other group ($n = 6$) received non-medicated food and water. Drug-treated and untreated control animals were sacrificed after 7 days of treatment, and plasma, urine, kidney, liver, spinal cord, and cerebral cortex specimens were collected from each group. Aliquots of the plasma or tissue were homogenized at 0°C with perchloric acid (final concentration, 0.4 M) and kept in ice for 10 min. The precipitate was removed by centrifugation, and a measured aliquot of the supernatant was neutralized with 4 N KOH–0.1 M KH_2PO_4 in the pH meter, keeping track of the volume of neutralization solution added. The neutralized mixture was frozen and then thawed; the precipitated potassium perchlorate was removed by centrifugation. The supernatant was evaporated to dryness, and the dry residue was redissolved in a small volume of water and clarified by centrifugation. Aliquots of the clarified supernatant were used for the quantitative determinations of the drug. The efficiency of the extraction procedure was validated by spiking untreated guinea pig plasma or tissue with L-653,180, extracting it as described above, and determining the identity and quantity of drug by high-performance liquid chromatography. Quantitation by this technique, however, was not accurate enough for amounts lower than 200 ng/ml, and thus the actual determinations of drug levels in tissue were performed by assaying inhibitory activity of extracts against the viral TK as described above. To eliminate the possibility that each tissue might have a different baseline due to inhibitory activity unrelated to the drug, a different standard curve was constructed for each tissue by adding known amounts of drug to samples of untreated control tissue and subjecting them to the same extraction and assay as the unknowns. The concentrations of the unknowns were computed from the percent inhibition and the equation for the respective standard curve.

Guinea pig model of genital HSV-2 infection. The vaginal closure membrane was ruptured by a moistened calcium alginate-tipped swab, and the animals were inoculated by intravaginal instillation of 5.7 $\log_{10}$ PFU of HSV-2, MS strain. Guinea pigs were examined daily for clinical evidence of primary genital herpes. As described elsewhere, genital HSV-2 infection in the guinea pig shares many features with genital herpes in humans, including the development of vesiculo-ulcerative skin lesions (16). On day 20 postinoculation (p.i.), animals that had exhibited primary genital skin lesions were randomized into two groups. Beginning on day 21 p.i., one group ($n = 10$) was administered L-653,180, ad libitum, in their diet (25 mg of drug/30 g of food) and drinking water (300 mg of drug/liter). The other group ($n = 10$)

TABLE 1. Concentration of L-653,180 in guinea pig tissues

Animal	Concn (ng/g or ng/ml) ^a				
	Spinal cord	Cortex	Plasma	Liver	Kidney
1	64	76	22	98	86
2	153	100	336	70	130
3	120	128	51	139	115
4	109	88	57	414	111
5	108	60	41	217	75
6	80	50	7	145	113
Mean $\pm$ SE	106 $\pm$ 13	84 $\pm$ 11	86 $\pm$ 51	180 $\pm$ 51	105 $\pm$ 8

^a The concentration of L-653,180 in pooled urine was 104 ng/ml.

received nonmedicated food and water. Drug therapy was continued to day 48 p.i. Animals were examined daily for evidence of recurrent herpetic disease, as described previously (17), during treatment and subsequently to day 91 p.i. by an observer blinded to the drug therapy.

Statistics. Data are expressed as mean $\pm$ standard error and were analyzed by Student's *t* test or Fisher's exact test.

RESULTS

The synthesis of L-653,180 (Fig. 1) proceeded efficiently from *cis*-1,2-cyclohexanedimethanol by monobenzylation and conversion of the remaining hydroxyl to a bromide-leaving group by standard methods. Subsequent alkylation of 2-amino-6-benzoyloxypurine (12) gave a mixture of 7- and 9-isomers, which were resolved chromatographically and characterized. NMR studies showed the ^{13}C shifts of the CH_2N , C^5 , and C^8 signals were consistent with those reported for 7- and 9-alkylated purines (8). Debenzylation and debenzoylation furnished the desired product (a racemic mixture of the 1'R, 2'S and 1'S, 2'R diastereomers).

Studies of guinea pigs treated for seven days with L-653,180 in their diet and water showed that the drug was readily accepted orally. Treated and untreated animals exhibited similar daily food and water consumption: food, 192 $\pm$ 18 versus 215 $\pm$ 9 g; water, 403 $\pm$ 35 versus 432 $\pm$ 23 ml, per group, respectively. In addition, treated animals showed similar weight gain during the treatment period (24.3 $\pm$ 5.1 g treated versus 23.3 $\pm$ 7.2 g untreated) and no adverse reaction to the drug was observed in any of the drug-treated animals.

After seven days, the animals were sacrificed and the concentrations of L-653,180 in urine, plasma and selected tissues were determined (Table 1). Drug was detected in all specimens from treated animals. The considerable variation in concentrations in plasma between animals was expected, since the time between last drug intake and sacrifice varied, and preliminary experiments had shown that the drug is rapidly cleared from plasma (4a). With the exception of results for one animal, the concentrations of drug in the liver specimens were similar. Likewise, there was little variability between animals with regard to drug concentrations in tissue. While the concentrations were low in all tissues, the mean values were similar to the *in vitro* IC_{50} s of L-653,180 against HSV-1 and HSV-2 TKs, which were both 0.27 μ M (75 ng/ml) under the assay conditions described in Materials and Methods. The IC_{50} of L-653,180 for HSV-1 TK with tritiated thymidine under lower substrate concentration (0.25 μ M) was 0.07 μ M (19 ng/ml). This lower substrate concen-

FIG. 2. Effect of L-653,180 on recurrent HSV-2 infections in guinea pigs. Drug was administered in food and water for 4 weeks beginning 21 days after intravaginal HSV-2 inoculation. Broken line, drug-treated group; solid line, untreated control group.

tration may more accurately reflect what is found in nondi-
viding neural tissue.

To determine the effect of a TK inhibitor on recurrent HSV disease, latently infected guinea pigs were given L-653,180 in food and drinking water for four weeks beginning day 21 p.i. The effect of L-653,180 on the frequency of recurrent genital herpes is shown in Fig. 2 and Table 2. Over a 10-week observation period, treated animals experienced a 33% reduction in the number of days when lesions were observed (mean lesion days, 10.3 versus 15.3 for controls, $P = 0.06$). During the four weeks of drug therapy (days 22 to 49 p.i.), treated animals experienced fewer recurrent lesion days, but the reduction was not statistically significant. However, in the 2-week period immediately following the cessation of drug administration, there was a significant reduction in recurrences ($P < 0.02$). This effect was not observed during the final 4 weeks of the experiment. Over the 10-week observation period, treated animals also showed a 36% reduction in severe recurrences (days when animals exhibited more than one recurrent lesion) (mean, 2.3 versus 3.6). Further, during the two weeks following completion of drug therapy fewer treated animals experienced severe re-

currences compared with untreated controls (1 versus 5, respectively; $P = 0.07$).

Animals exhibited no adverse reactions to the drug either during treatment or during the washout period.

DISCUSSION

The TK inhibitor (L-653,180) was prepared by a five-step convergent synthesis and shown to be an effective inhibitor of HSV TK in vitro. Molecular modelling studies were undertaken to superimpose L-653,180 (both the 1'R, 2'S and the 1'S, 2'R diastereomers) with an X-ray structure-based conformational model of thymidine (21). Superimposition of the heterocyclic amide function of the two heteroaromatic systems allowed the hydroxyl group of L-653,180 to approach the 3' hydroxyl position of thymidine, but not the 5' hydroxyl position. This preliminary observation may serve to explain why the racemic compound inhibits, but is not a substrate for, the thymidine-binding enzyme.

Viral TK inhibitors have been shown to interfere with the reactivation of latent HSV from ganglia and cell culture systems (1, 10, 14). Inhibition of the reactivation of latent

TABLE 2. HSV-2 recurrences in guinea pigs

Group (n)	Mean ($\pm$ SE) lesion days ^a during p.i. days					
	22-35	36-49	50-63	64-77	78-91	22-91
Untreated (10)	5.2 (0.9)	3.2 (0.7)	3.1 (0.7)	1.9 (0.7)	1.9 (0.6)	15.3 (2.6)
Treated (10)	3.7 (0.9)	2.4 (0.4)	1.1 (0.5)	1.5 (0.5)	1.6 (0.5)	10.3 (1.5)
<i>P</i> value	0.13	0.17	0.02	0.34	0.35	0.06

^a Lesion days, no. of days when lesions were observed.

virus in vivo would be expected to prevent herpetic recurrences. The first evidence that a selective TK inhibitor could prevent recurrent HSV disease was recently reported by Kaufman et al. (5). They demonstrated that treatment with 5'-ethynylthymidine reduced spontaneous ocular HSV-1 recurrences in latently infected squirrel monkeys. With a different compound (9-[[*(Z)*-2-(hydroxymethyl)cyclohexyl]methyl]guanine) and animal model (spontaneous genital HSV-2 recurrences in latently infected guinea pigs) our data provides the second in vivo demonstration that TK inhibitors can reduce recurrences. The guinea pig model of genital herpes has proven useful in evaluating putative antiviral drugs. For example, this model successfully predicted the efficacy of acyclovir and foscarnet (phosphonoformate) in the treatment of genital herpes (7, 13). Thus, our initial studies suggest that chronic administration of a TK inhibitor might be expected to reduce HSV recurrences in humans.

The effect of L-653,180 on recurrent genital herpes was nominal. The poor solubility of L-653,180 limited the amount of drug that could be delivered in drinking water. Thus, to increase the dose of drug administered, additional L-653,180 was incorporated into the food. After 7 days of drug administration, concentrations in tissues were greater than the in vitro IC_{50} for HSV TK. Treatment for 28 days resulted in a modest decrease in genital recurrences, with the greatest reduction observed in the 2-week period following completion of drug therapy. This delayed effect may indicate that drug is accumulated in sensory ganglia over time until a concentration sufficient to result in the in vivo inhibition of viral TK is achieved. After day 64 the effect was lost, possibly as the drug was eliminated from ganglia.

The limited clinical response may reflect inadequate inhibition of viral TK in vivo by orally administered L-653,180. These results suggest either that the IC_{50} of L-653,180 in vivo is higher than that in vitro (which is unlikely in view of the fact that thymidine concentration in neural tissue is much lower than that used in in vitro determinations) or that greater than 50% inhibition of the enzyme is required to effect a significant reduction in recurrences and that continued therapy is needed to reach that concentration. Support for the second possibility can be found in studies by Coen et al. (2) with HSV-1 mutants that produce over 90% less TK than the wild type but which are able to reactivate as efficiently from latently infected ganglia by cocultivation. Further studies are necessary to quantify the relationship between TK inhibition and prevention of HSV recurrences. More potent and bioavailable TK inhibitors might be expected to produce greater control of recurrent herpetic disease. The results presented here, however, suggest that selective inhibition of HSV TK can reduce recurrent episodes. Further studies of this novel approach to the control of recurrent disease are warranted.

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