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Specific Inhibitors of Herpes Simplex Virus Thymidine Kinase Diminish Reactivation of Latent Virus from Explanted Murine Ganglia

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Two specific inhibitors of herpes simplex virus thymidine kinase, N^2-phenyl-2′-deoxyguanosine and N^2-(*m*-trifluoromethylphenyl)guanine, were tested for their ability to inhibit the reactivation of virus from explant cultures of latently infected murine trigeminal ganglia. Both compounds significantly diminished the frequency of reactivation compared with that of untreated controls.

Herpes simplex virus (HSV) exhibits a multistage life cycle within mammalian hosts. After primary lytic infection in peripheral mucocutaneous tissue, the virus can enter nerve terminals, spread by axonal transport, and establish lifelong latent infections in sensory ganglia (19). During latency, no infectious virus is detectable, and viral gene expression is very restricted (16). Latency may occasionally break down, leading to the production of infectious virus and the initiation of a reactivation event.

We and others have recently shown that although thymidine kinase (TK)-negative HSV mutants fail to reactivate after explant culture, they do express the latency-associated transcripts in sensory neuronal nuclei (2, 11, 18) and can be reactivated by superinfection-rescue with complementing virus (2, 4). These data demonstrate that TK is not required for establishment of latency and suggest that TK is required for reactivation.

The apparent requirement for TK in animal models of latency and pathogenesis has led to efforts to develop anti-HSV drugs that inhibit HSV TK specifically (reviewed in reference 15). Two such drugs are N^2-phenyl-2′-deoxyguanosine (PhdG) and N^2-(*m*-trifluoromethylphenyl)guanine (*m*-CF_3PhG) (6, 7, 15). These compounds are N^2-substituted guanines, a family of compounds that can inhibit TK by competition with the enzyme substrates thymidine and deoxycytidine (15). Both PhdG and *m*-CF_3PhG have demonstrated potent and selective inhibition of HSV TK in vitro (6, 7), and PhdG has been shown to inhibit HSV TK in HeLa cells and exhibit minimal cytotoxicity (6). *m*-CF_3PhG also appears to be minimally cytotoxic; at 133 μM it had no detectable effect on the growth or morphology of HeLa cells, and neither it nor PhdG affected the morphology of Vero cells at concentrations up to 300 μM (unpublished results).

In this study we used PhdG and *m*-CF_3PhG in an animal model of HSV latency to test whether the selective inhibition of TK observed in vitro could translate to a selective inhibition of reactivation from explant cultures of latently infected murine trigeminal ganglia. These experiments allowed us to assess further the role of TK in the reactivation of viral latency and to test the potential of these compounds as antiviral agents.

Procedures for the growth and assay of the KOS strain of HSV type 1 have been previously described (14). CD-1 mouse embryo cells and African green monkey kidney (Vero) cells were prepared, propagated, and maintained as previously described (8, 13). Single-cycle growth assays were performed at 37°C as previously described (8) with the addition of drug to cultures after virus adsorption for 1 h. Infected cells were harvested 20 h postinfection, and the yields of virus were assayed on Vero cells.

Seven-week-old randomly bred CD-1 mice (Charles River Laboratories, Kingston, N.Y.) were anesthetized with sodium pentobarbital; corneas were scarified, and the mice were inoculated with 2×10^6 PFU of KOS per eye in a volume of 20 μl as described previously (17). Assays of latent infection by ganglionic cocultivation were performed on day 30 as previously described (10), with the addition of 150 μM PhdG or *m*-CF_3PhG to explant cultures where appropriate.

The results of the reactivation experiments indicated that both PhdG and *m*-CF_3PhG dramatically reduced the number of latently infected trigeminal ganglia that yielded virus upon explant culture. Control explant cultures (with no drug) yielded virus from 61% of ganglia, whereas a concentration of 150 μM PhdG or *m*-CF_3PhG was sufficient to reduce the frequency of reactivation to 15 and 5%, respectively (Table 1). These reductions in reactivation were significant ($P < 0.005$ by the F test). This suggested that the two compounds specifically inhibited reactivation from latently infected neurons. A potential alternate explanation for this result was that the compounds did not inhibit reactivation per se but rather prevented the growth of virus in adjacent murine fibroblasts and/or in the Vero cells used to enhance detection of reactivation. However, the yield of HSV type 1 wild type (KOS) from Vero cells was unaffected by the addition of 150 μM PhdG or *m*-CF_3PhG (Table 1). Concentrations of either drug up to 300 μM similarly had no effect on plaque formation in Vero cells (data not shown). Growth of KOS in CD-1 mouse embryo fibroblasts was inhibited by 150 μM PhdG and *m*-CF_3PhG in that three- to fivefold reductions in virus yield were observed compared with untreated KOS

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TABLE 1. Effect of TK inhibitors on reactivation from explanted ganglia and on yield from cell culture of wild-type HSV

Drug	No. of ganglia reactivated/ explanted	PFU/cell[a]	
		Vero	CD-1 mouse
Control (no drug)	11/18	180	22
PhdG (150 μM)	3/20	180	6.2
m-CF_3PhG (150 μM)	1/20	180	4.2

[a] Infected at a multiplicity of five. Results are the averages of two experiments.

yields (Table 1). This reduction in yield, however, did not cause a net loss of virus; thus, it would not be sufficient to prevent detection of reactivated virus from the ganglion explants. Our interpretation of the results is that the compounds directly inhibit reactivation from ganglionic neurons.

These results are consistent with previous suggestions that viral TK plays a pivotal role in reactivation (2, 4, 11, 18). Although the use of specific TK inhibitors did not completely prevent reactivation, it significantly decreased the number of ganglia yielding virus, mimicking the decreased reactivation phenotype of TK-deficient viruses (2, 4, 5, 11, 17, 18). Although doses much greater than the concentration required for 50% inhibition of HSV TK were used (6, 7), it may be that almost complete extinction of HSV TK activity is necessary to prevent reactivation. This would be consistent with previous observations that less than 10% of wild-type TK activity is sufficient to allow wild-type reactivation in this mouse eye model (1). It is possible, therefore, that the doses of drugs used in this study were insufficient to totally abolish TK activity.

The mechanism by which HSV TK functions during reactivation is not clear. TK can phosphorylate deoxypyrimidine nucleosides and functions in cultured cells, at least in part, to increase the pool of nucleotides available for viral DNA synthesis (12). Viral TK is not important in actively dividing cells, as previously shown by Dubbs and Kit (3). This study confirms this finding in that TK inhibitors had little or no effect on wild-type virus yields, and the TK-negative virus *dl*sptk (2) gave wild-type yields in Vero and mouse cells (data not shown). TK may, however, be particularly important in nondividing cells (5, 9) such as neurons, where such host functions may be deficient or absent. It will be interesting to determine whether the effects of the TK inhibitors on reactivation are reversible and whether they lead to specific blocks in viral gene expression.

The very significant reduction in reactivation from ganglia treated with PhdG or *m*-CF_3PhG seen in this study suggests that these compounds or other specific inhibitors of HSV TK may have potential clinical application, especially in the prevention of HSV reactivation, for example, during immunosuppressive treatments in which herpetic reactivations can cause serious morbidity and mortality.

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