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Ultratrace Determination of Penciclovir-triphosphate (PCV-TP) in a Herpes Simplex Virus Type 1 (HSV-1)-Infected Cell Line using Flow-Injection Electrospray Ionization Tandem Mass Spectrometry (MS-MS). E.M. Smyrnis, R. Burton, F. Abbott, R.A. Wall, and S.L. Sacks. Viridae Clinical Sciences Inc. and University of British Columbia, Vancouver, Canada.

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; [PCV]; BRL39123) is a potent and specific antiviral agent with efficacy against both HSV and varicella zoster virus (VZV). A key metabolic advantage of this agent may be its prolonged half-life as PCV-TP in infected cells. To further study the pharmacokinetics at the intracellular site of activity *in vivo*, it would be advantageous to detect low levels of PCV-TP in cells. To this end, an MS-MS method was devised. Confluent monolayers of Schwannoma (SW) cells were infected with KOS (wild type) or viral thymidine kinase-negative [TK^-] HSV-1 (ACG^r4). PCV (1 μM to 10 μM) was added for 6 h at 20 h post-infection. Monolayers were washed in 10 ml PBS and extracted in 3 ml of ammonium formate (20 mM), dimethylhexylamine (2 mM) and methanol (to 70%) buffer (pH 6.0). 20 μL of extract was delivered in a continuous stream (50 μL/min) of 50% isopropyl alcohol/0.05% NH_4OH to the electrospray interface. Tandem ESI (in the negative ion mode)/mass spectrometry was used to determine the presence of a 492 to 158.1 transition fragment, indicative of PCV-TP.

	Uninfected	KOS (moi=1)	KOS (moi=10)	ACG^r4 (moi 1)
Untreated*	<LD[‖]	<LD	<LD	<LD
PCV (1 μM)	<LD	2,440 ± 240[†]	4,150 ± 1,370	ND[#]
PCV (10 μM)	<LD	11,500 ± 1,280	14,600 ± 780	<LD

* Controls included uninfected/PCV-treated cells (PCVTP<LD); [‖] LD = limit of detection (1,250 ± 310 pmol/10^6 cells); [†] expressed in pmoles/10^6 cells; [#] ND = not done

The MS-MS assay showed that PCV-treated, wild type HSV-1-infected cells made PCVTP at high concentration, in contrast to the control cells. This technique may allow quantitation of minute levels of PCV-TP from cells to more accurately determine intracellular pharmacokinetics.

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Famciclovir and valaciclovir treatment during acute HSV-1 infection of mice have markedly different effects on subsequent virus reactivation from ganglia. HJ Field & AM Thackray, University of Cambridge, Madingley Road, Cambridge, CB3 OES, U.K..

This study was designed to investigate the effects of two nucleoside analogue prodrugs: famciclovir (FCV) and valaciclovir (VACV), on the prevention of HSV-1 latency in mice. A cutaneous infection was established by inoculation of the ear pinnae of BALB/c mice with HSV-1 (SC16). Antiviral treatment was initiated on days 1, 2, 3, 4, or 5 post infection (p.i.), and all therapy ceased on day 10 p.i. The compounds were administered *ad libitum*, in the drinking water, at 1mg/ml (approximately 100 mg/kg/day). The responses to therapy (weight gain, visible lesions, inflammation, mortality and reduced virus titres in the target organs), all indicated that FCV was superior to VACV and FCV treatment led to the rapid and complete eradication of infectious virus from skin and neural tissues during the acute phase of the infection. Moreover, on cessation of VACV therapy, there was a recurrence of infectious virus in the brainstem in all VACV-treated groups. No recurrence of infectious virus was observed, however, when FCV treatment was stopped. Similar results were obtained when compounds were administered twice daily by gavage at 50 mg/kg per dose. Four months later, latent virus could be reactivated from ganglia explants (ipsilateral and contralateral trigeminal and dorsal root) from all of 16 control mice. Latent virus was not reactivated from the ganglia of FCV-treated mice except ipsilateral ganglia, and only when the start of therapy was delayed until days 4 p.i. (2/16) or 5 p.i. (6/16). By contrast, virus was reactivated from all VACV-treated groups, even when therapy commenced on day 1 p.i. (13/16). These experiments confirm that VACV and FCV have different effects on the pathogenesis of HSV in the mouse that cannot be accounted for by differences in plasma pharmacokinetics or potency in murine cells. We suggest that these data have important implications for HSV latency in man.