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Identification of the Specific Serine Residue at the Active Site of the Herpes Simplex Virus Type 1 Protease.

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Herpes simplex virus type 1 (HSV-1) protease is responsible for proteolytic processing of itself and of the nucleocapsid associated protein, ICP35 (infected cell protein 35) [Liu and Roizman (1991) *J. Virol.* **65**, 5149-5156]. A temperature sensitive mutation in the gene encoding the protease affects processing of ICP35 and results in failure of nucleocapsids to package DNA. Inhibitor studies indicated that the HSV-1 protease is sensitive to the serine protease inactivator, diisopropyl fluorophosphate (DFP), and therefore, was investigated further. The irreversible inactivation by DFP is dependent on time and concentration of DFP. Saturating amounts of a peptide substrate protected the HSV-1 protease from the inactivation by DFP whereas a peptide which is not a substrate did not protect against the inactivation indicating that the modification occurs at the active site. Loss of activity correlates linearly with the incorporation of [^{3}H] DFP and extrapolation to zero activity indicated that 0.7 [^{3}H] DFP molecules reacted per active site. Analysis of inactivated protease by mass spectrometry indicated a stoichiometry of 1 DFP per molecule protease. In order to identify the spectific residue modified by DFP, the protease was labeled with [^{3}H] DFP and subsequently digested with chymotrypsin. The resulting peptides were separated by reverse phase HPLC and 80 % of the radioactivity was contained in one peptide. Sequencing analysis by mass spectrometry identified the active site serine as the residue modified by DFP. This residue and the region in which it is found is highly conserved among the herpes viral proteases. This data demonstrates that the HSV-1 protease is a serine protease.

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Comparative activity of penciclovir and acyclovir against herpes simplex virus type 2 in cell culture. T.H. Bacon, R. Standring-Cox, B.A. Howard, S. Brooks, G.L. Lambert and M.R. Boyd. SmithKline Beecham Pharmaceuticals, Great Burgh, Epsom, Surrey, KT18 5XQ, U.K.

The activity of the potent and selective antiherpesvirus agent penciclovir (PCV) against herpes simplex virus type 2 (HSV-2) has been compared with that of acyclovir (ACV). In plaque reduction assays PCV was more active than ACV in WISH and WI-38VA13 cells, the 50% effective concentrations (EC_{50}s) for PCV compared with ACV were 0.35μg/ml vs 0.74μg/ml in WISH cells and 0.8μg/ml vs 2.9μg/ml in WI-38VA13 cells. EC_{50}s were not significantly different in Hs68 and WI-38 cells but were higher for PCV (2.1μg/ml) than ACV (0.9μg/ml) in MRC-5 cells. In virus yield assays PCV was a more potent inhibitor of viral replication than ACV in MRC-5 cells infected with HSV-2 at 0.01pfu/cell and treated continuously for 72 hours. For example, at 3 and 1μg/ml the virus yield was reduced by 100 and 97% respectively for PCV, compared with 76 and 85% respectively for ACV (mean values from 3 strains). In MRC-5 cells infected with HSV-2 at 0.0001pfu/cell treated with 6 hour daily pulses of compound for 3 days, PCV was also more active than ACV. In MRC-5 cells infected at 1pfu/cell and treated with a single 2 hour pulse at 1.6μg/ml, the virus yield at 24 hours was reduced by 83% for PCV compared with 47% for ACV. In pulse treated cultures there was a more effective inhibition of viral DNA synthesis by PCV than ACV. The persistent antiviral activity of PCV may be explained by the longer intracellular half-life of PCV-triphosphate (20 hours) compared to ACV-triphosphate (1 hour) in HSV-2 infected MRC-5 cells (1). In conclusion, the relative potencies of PCV and ACV in plaque reduction assays were dependent on the cell line. Although PCV was less active than ACV in the plaque reduction assay in MRC-5 cells, PCV was more potent than ACV in virus yield reduction assays in MRC-5 cells infected at 0.01pfu/cell with HSV-2 when compounds were present continuously. When cultures were pulse treated to give condidtions more representative of the clinical siutation PCV gave a more prolonged inhibition of virus replication than ACV. (1) Earnshaw, D.L. et al (1992) Antimicrob. Agents Chemother. 36, 2747-2757.