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Recurrent and prolonged herpes simplex encephalitis

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Recurrent herpes simplex virus(HSV) encephalitis is becoming more common, despite intensive early acyclovir therapy. We report several cases of recurrent or prolonged HSV encephalitis, in which HSV genome reappeared or persisted for over 1 month. Patients & methods: Between 1986 & 1995, there were 22 patients with HSV CNS infections; 17 cases of acute encephalitis including 4 cases of limbic encephalitis,3 of brainstem encephalitis, and 5 of meningitis. Diagnosis of HSV CNS infections was based on significant changes in serum/CSF ratio of ELISA or FA antibody titres and PCR study in CSF. PCR was carried out by microplate hybridization of PCR amplified DNAs for HSV UL44 region, except for several cases which were done by direct PCR using HSV polymerase gene or thymidine gene. Results: In most cases, acyclovir (30mg/kg per day) was given for 10-14 days. 2 cases suffered a recurrence, and 5 cases including brainstem encephalitis had prolonged courses. In these cases, HSV detection by PCR persisted for over 1 month after onset of symptoms. In 2 out of 5 cases of HSV meningitis, HSV genome was also detected over 1 month after onset. For recurrent and prolonged encephalitis, acyclovir therapy was repeated for 2 weeks, and ara-A was added. In 3 cases, ara-A was definitely effective.

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Cyclic HPMPC (cHPMPC) is Hydrolyzed to Cidofovir (CDV) by an Intracellular cCMP Phosphodiesterase. D.B. Mendel, T. Cihlar, K. Moon, and M.S. Chen. Gilead Sciences, Foster City, CA, U.S.A.

cHPMPC is an intracellular prodrug of CDV (HPMPC; 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl] cytosine), an acyclic phosphonate analog of dCMP with potent activity against a broad spectrum of herpesviruses including cytomegalovirus (CMV). CDV has been shown to exert a dose-dependent anti-CMV effect as measured in the urine and semen of advanced AIDS patients, and to retard the progression of CMV retinitis in these patients. cHPMPC and CDV have comparable anti-CMV activity *in vitro* and in animal models, but cHPMPC has shown substantially reduced toxicity in several animal species. We now report that cHPMPC is hydrolyzed to CDV by a cellular cCMP phosphodiesterase which hydrolyzes a variety of substrates including cAMP and cCMP. cHPMPC is a competitive inhibitor of the natural substrates, and is accepted as a substrate by the enzyme purified from human liver. The Km (250 μM) and Vmax (25 fmol/min·μl) for cHPMPC are similar to those for the natural substrates cCMP (75 μM; 88 fmol/min·μl) and cAMP (23 μM; 44 fmol/min·μl). cHPMPC is readily hydrolyzed to CDV in all primary human cell systems tested, including those derived from target organs that might be infected in a patient with disseminated disease, and its hydrolysis is not altered in CMV infected cells.

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Point Mutation in the DNA Polymerase Gene of a Clinical Cytomegalovirus Isolate which was Cross-Resistant to GCV and (s)-HPMPC

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Both ganciclovir(GCV)-sensitive and -resistant cytomegaloviruses(CMV) were isolated from the patient with CMV retinitis and encephalitis. GCV-resistant mutant was also cross-resistant to (*S*)-1-[(3-hydroxy -2-phosphonylmethoxy)prophyl]cytosine (s-HPMPC). Both CMVs showed the same restriction cleavage pattern when digested with *Eco* RI, *Hin* dIII, or *Pst* I, showing that the cross-resistant mutant developed during the long term treatment with GCV. SSCP analysis suggested that the mutation exists in the DNA polymerase gene, but not in the UL97 phosphotransferase gene. When 386 bp PCR product was sequenced, it was revealed that the first base of the coden 501(CTC) changed from C to T, resulting in the repalcement of Leucine with Phenylalanine. The mutation of coden 501 also results in the loss of an *Alu* I restriction site (recoginition sequence AGCT) in 386 bp PCR product. Therefore, GCV-resistant CMV based on the coden 501 was easily distinguished from the sensitive one by the digestion of 386 bp PCR product with *Alu* I.

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Establishment of Assay system for Screening of Human Cytomegarovirus UL80-Proteinase Inhibitor

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The proteolitic processing of the human cytomegarovirus (HCMV) assembly protein is an essential step in virion maturation. The viral specific proteinase responsible for this cleavage is the amino-terminal half of the protein encoded by UL80 open reading frame. Therefore it was considered that the proteinase is one of the target enzyme for antiviral agents.

To establish *in vitro* assay system for screening of the proteinase inhibitor, the HCMV UL80 proteinase was expressed as the form of fusion protein with maltose-binding protein(MBP) in *Escherichia coli* and purified by affinity chromatography using amyrose resin. Using a synthetic peptide of mimics M site cleavage as substrate, we assayed proteinase activity by HPLC. We proved that protein expressed in *E.coli* has HCMV specific proteinase activity. The characterization the MBP-UL80 proteinase was demonstrated by determination of a kinetic optimum pH 7.2 and incubation temperature(37°C).

Our results indicated that MBP-UL80 proteinase expressed in *E.coli* is useful as a source of the enzyme on *in vitro* assay system for screening HCMV proteinase inhibitors.