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SCID-hu Thy/Liv Mouse Model for In Vivo Human CMV Antiviral Evaluation. C. A. Stoddart, M. R. Bogan, M. E. Moreno, V. D. Linquist, S. Warren, and J. M. McCune. Gladstone Institute of Virology and Immunology, UCSF, San Francisco, CA, USA.

A SCID-hu Thy/Liv mouse model has been developed and refined for in vivo evaluation of antivirals against wild-type and drug-resistant human CMV clinical isolates. Immunodeficient C.B-17 *scid/scid* mice are implanted with human fetal thymus and liver underneath the kidney capsule and infected by direct inoculation of the implants with 10^4 to 10^6 plaque-forming units (PFU) of CMV. For CMV (Toledo), peak implant viral titers of 10^7 PFU and viral DNA load of 10^9 copies per gram are attained by 11 days after inoculation. Viral replication in the implants is maintained at this level for at least 60 days. Twice daily administration of intraperitoneal ganciclovir or foscarnet caused statistically significant and dose-dependent reductions in implant viral load. In mice treated postexposure with ganciclovir, CMV titers were reduced 250-fold compared to untreated mice 11 days after inoculation. The long-term viral replication that occurs in the human tissue implants should facilitate study of the development of antiviral drug resistance in vivo, and experiments are underway to further develop the model for this purpose. Results from experiments in which mice are inoculated with drug-sensitive CMV, treated with escalating low-dose ganciclovir by subcutaneously implanted mini-osmotic pump, and periodically evaluated for the presence of drug-resistant CMV mutants will be discussed.

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Cyclic HPMPC is safe and highly effective against guinea pig cytomegalovirus infection in immunocompromised animals. N. Bourne, M.Hitchcock and DI Bernstein. Childrens Hospital Research Foundation, Cincinnati, OH, and Gilead Sciences Inc, Foster City, CA.

HPMPC has proven effective in the treatment of herpes virus infections but associated toxicity has limited its clinical applications. The compounds cyclic derivative (CHPMPC) also has antiviral activity against herpesviruses and is less toxic than the parent compound. In the studies described here we have evaluated the safety and efficacy of CHPMPC against cytomegalovirus (CMV) infection in an immunocompromised guinea pig model. Initially we evaluated CHPMPC's toxicity in both immunocompetent and immunocompromised animals. The drug was administered by IP injection at either 5mg/kg/day for 7 days q.d. (total dose 35mg/kg) or as 2 doses of 17.5mg/kg. Neither treatment regimen produced hematologic (WBC, Hgb, and HcT), hepatic (SGPT, bilirubin and albumin) or renal (BUN and creatinine) toxicity when given alone or in combination with cyclophosphamide (CY). Having established that the compound was not toxic we next evaluated the efficacy of the two treatment regimens against guinea pig CMV (gpCMV) infection. Forty seven male Hartley guinea pigs (300-400g) were immunocompromised by an initial IP injection of 100mg/kg CY with a second dose of 50mg/kg administered given 7 days later. One day after the initial dose of CY animals were inoculated by subcutaneous injection with 4.80 $\log_{10}$ pfu of a virulent salivary gland passaged gpCMV. Antiviral therapy was initiated twenty four hours after virus inoculation with animals receiving CHPMPC daily (N=16), in two doses (N=16) or placebo (N=15). Thirteen placebo treated animals died compared to one in the daily treated group and none in the group that received drug in 2 doses ($p < 0.0001$). Thus CHPMPC is both non toxic and highly effective against CMV infection in this model. Further studies with this compound appear warranted.

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Development of An Elisa Assay for Determining Antiviral Activity Against Epstein-Barr Virus Replication. G. Marshall and E.R. Kern. Univ. of Alabama School of Medicine, Birmingham, Ala., USA

Since Epstein-Barr Virus (EBV) does not undergo a productive infection in tissue culture cells, evaluation of new antiviral agents has utilized complex, labor intensive assays for quantifying genomic copies or viral antigens. We have recently developed a labor-saving ELISA for determining the effect of antiviral drugs on the expression of viral casid antigen (VCA) in cell lines superinfected with EBV. The ELISA assay eliminates hours of microscopy required to perform a standard immunofluorescence (IF) or hybridization assay. Following virus superinfection and incubation with drug, cells were counted, spotted on slides for IF or in situ hybridization and aliquots added in triplicate to a 96-well tissue culture plate for ELISA. The efficacy of selected antiviral drugs with known activity (Acyclovir, Penciclovir, Ganciclovir, Cidofavir, Foscarnet, and Retrovir) against EBV replication in Daudi cells superinfected with P3HR-1 virus was determined by IF, in situ DNA hybridization, and ELISA and are summarized as EC_{50} (μg/ml) values in the table.

	ACV	PCV	GCV	AZT	PFA
IFA	1.57	0.61	1.52	1.80	0.36
DNA	1.01	1.03	0.30	1.45	9.49
ELISA	1.91	1.29	8.07	1.46	3.27

The results in the ELISA assay correlated highly with both IF and in situ hybridization. Thus, the ELISA assay can be a useful labor-saving assay for screening new antiviral agents for antiviral activity against EBV replication.

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Activity of benzimidazole riboside 1263W94 (5,6-dichloro-2-(isopropylamino)-1-β-L-ribofuranosyl-1*H*-benzimidazole) against Epstein-Barr Virus

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The latently infected EBV lymphoblastoid cell line Akata is efficiently induced to lytic replication by crosslinking of surface immunoglobulin. We analyzed linear, replicative and episomal forms of EBV DNA following induction with the use of terminal probe analysis and pulsed field gel electrophoresis followed by Southern blot hybridization. Upon induction we detected ladder arrays of linear EBV genomes indicative of viral replication, as well as a twenty-fold increase in viral DNA. The generation of linear and high molecular weight DNA was sensitive to L-ribofuranosyl benzimidazole compounds previously described as inhibitors of HCMV DNA replication. The IC_{50} for benzimidazole 1263W94 was 0.8 μM, and EBV DNA replication was completely inhibited at 10 μM. EBV early antigen (EA-D) induction was not inhibited by the drug indicating that inhibition by 1263W94 did not occur at an early time after induction. D-ribofuranosyl benzimidazole compounds that act as inhibitors of HCMV DNA maturation, including BDCRB (5,6-dichloro-2-bromo-1-β-D-ribofuranosyl-1*H*-benzimidazole),did not affect the accumulation of high molecular weight or monomeric forms of EBV DNA in the induced cells. Phase I pharmacokinetic studies show that 1263W94 is orally bioavailable and nontoxic in humans at 400 mg single doses, which generated serum concentrations >10 μM. Therefore the compound 1263W94 is a potential therapy for prevention of EBV positive post-transplant lymphomas and other EBV-associated diseases of transplant recipients and persons with AIDS.