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Interleukin-12 is a novel antiviral therapy

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The therapeutic and prophylactic antiviral efficacy of interleukin-12 (IL-12) was studied using murine models of herpes simplex virus (HSV) and murine cytomegalovirus (mCMV) infection. Therapeutic intraperitoneal administration of IL-12 commenced 6 hours after mice were infected with HSV, and was continued daily for a total of 5 days. IL-12 therapy improved the survival rates of mice with systemic HSV infection, compared with that of placebo treated infected mice. Subcutaneous administration of IL-12 also improved the rate of survival of systemic HSV infection, although higher doses were required to give comparable effects. Prophylactic IL-12 produced the greatest effect on survival of an otherwise lethal systemic infection. Intraperitoneal administration of IL-12 for 2 days before and 3 days after systemic infection with HSV permitted over 80% of mice to survive the infection. These surviving mice were resistant to subsequent re-infection with HSV. Such resistance was apparently specific for HSV infection, since a second group of survivors succumbed to a lethal infection of mCMV. Infectious virus could not be recovered from 5 day cultured lumbar ganglia explants, dissected from survivors of IL-12 prophylaxis, suggesting that IL-12 treatment reduced the establishment of latent HSV infection. Mice treated with IL-12 showed enhanced Natural Killer (NK) cell activity *in vitro*. MCMV infection induced a short-lived NK cell response which could be prolonged by *in vivo* IL-12 treatment.. One action of IL-12 may therefore be to enhance NK cell mediated clearance of the virus. However, IL-12 therapy was also effective in mice carrying the *beige* mutation (which reduces NK cell lytic activity) and in mice depleted of NK cells using anti-asialo antibody, indicating that IL-12 has additional activities *in vivo*.

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(E)-2'-Deoxy-(fluoromethylene) Cytidine potently inhibits Replication of Human Cytomegalovirus Strains both Sensitive and Resistant to Ganciclovir and/or Foscarnet and Potentiates Antiviral Effects of Ganciclovir.

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(E)-2'-Deoxy-(fluoromethylene) cytidine (MDL 101,731) is a novel inhibitor of cellular ribonucleotide reductase (RRI) that is much more a potent antitumor agent than classical RRIs such as hydroxyurea. Cellular RRIs may also inhibit replication of human cytomegalovirus (HCMV); however, RRIs yet tested show only low antiviral selectivity. We observed antiviral activity of MDL 101,731 against several HCMV strains in cultures of human foreskin fibroblasts (HFF). In the plaque reduction assay, the concentration required to inhibit 50% of virus growth (IC_{50}) ranged for different virus strains from 12 to 38 nM. Similar IC_{50} values were obtained when effects of MDL 101,731 on HCMV DNA synthesis were measured. MDL 101,731 at a concentration of 200 nM completely suppressed production of infectious virus in HFF. The concentration required to inhibit 50% of viable cells (CC_{50}) in confluent HFF (used for antiviral assays) was 60 µM while CC_{50} was 850 nM in growing HFF. Therapeutic index (ratio CC_{50}/IC_{50}) was at least 1000 and 20 in confluent or growing HFF, respectively. MDL 101,731 was active against ganciclovir resistant clinical isolates with specific mutations in UL 97 kinase gene and ganciclovir/foscarnet clinical isolates with specific mutations in UL 97 and UL 54 DNA polymerase genes. MDL 101,731 has synergistic antiviral effects in combination with ganciclovir. The results show that MDL 101,731 is a highly selective inhibitor of HCMV replication and also potentiates antiviral effects of ganciclovir.

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In Vivo Anti-Cytomegalovirus (CMV) Activity and Safety of Oral Lobucavir in HIV Infected Patients.

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Lobucavir is a cyclobutyl analog of guanine with broad spectrum *in vitro* activity against most herpes-type viruses, Hepatitis B and HIV. Oral Bioavailability approaches 40% and single doses up to 800mg have been well tolerated. This dose-escalating pilot study evaluated the *in vivo* anti- CMV activity and safety of oral lobucavir at doses of 200mg BID, 200mg QID and 400mg QIDfor 28 days in individuals dually infected with HIV and CMV. Antiviral CMV was measured by eradication of CMV viruria and reduction in CMV titer in semen. All doses were well tolerated without drug-related clinical or laboratory adverse events including absence of myelosuppression. Anti-CMV activity, in terms of % losing CMV viruria and log reduction from baseline PFU/ml in semen was demonstrated:

Dose level	Day 1		Day 14		Day28	
200mg BID	100%[1](n=6)	0[2](n=1)	83%	-0.09[2]	60%	-0.70[2]
200mg QID	100%(n=10)	0(n=6)	56%	-0.49	44%	-0.50
400mg QID	100%(n=7)	0(n=6)	86%	-0.57	43%	-1.09

[1]% + CMV viruria [2]Mean $\log_{10}$ change from baseline PFU/ml semen. Lobucavir is a well-tolerated, orally bioavailable broad-spectrum antiviral agent with anti-CMV activity. Further studies in CMV disease and prophylaxis are warranted.

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2-Hydroxymethylcyclopropylidenemethylpurines and -pyrimidines - New Nucleoside Analogs with a Broad Spectrum Antiviral Activity.

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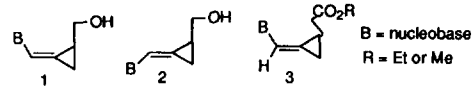
New nucleoside analogs 1 and 2 comprising a rigid cyclopropylidene-methyl moiety were synthesized. Alkylation of nucleic acid bases with ethyl E- and Z-2-bromo-2-bromomethylcyclopropane carboxylates followed by elimination of HBr gave E- and Z-esters 3. Reduction with diisobutyl-



aluminum hydride led to the title compounds 1 and 2. The Z-analogs 1 (B = Ade, Gua, Cyt) were stable at pH 1 and analogs 1 and 2 (B = Ade) were substrates for adenosine deaminase. Deamination of 1 (B = Ade) exhibited a distinct preference for (+)-enantiomer. The Z-analogs 1 (B = Ade, Gua, Cyt) were potent inhibitors of the replication of HCMV *in vitro* (IC_{50}'s = 0.04 - 3.4 µM) at non-cytotoxic concentrations. Analog 1 (B = Thy) and all of the E-analogs 2 were inactive or weakly effective. Several of the analogs including those active against HCMV also were effective against HSV-1, HSV-2, VZV, EBV, HHV-6 and HIV-1 at non-cytotoxic concentrations. Certain Z-analogs were active *in vitro* against murine CMV. In mice infected with murine CMV, the Z analogs (B = Ade, Gua) were highly effective in providing protection from mortality at concentrations (5.6 to 50 mg/kg/day) comparable to ganciclovir. Supported by NIH grants RO1-CA32779, U19-AI31718, RO1-AI33332 and NO1-AI-65290.