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Dual therapy with IL-12 and IFNα provides a novel approach to the management of chronic viral disease.

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Interferon-alpha (IFNα) and Interleukin-12 (IL-12) were used in combination in experimental models of virus infection, to examine the hypothesis that dual cytokine therapy is more effective in controlling viral disease, than is either single agent. A primary indication for such a combination therapy might be in the management of Hepatitis B and C infection. In the absence of animal models of hepatitis infection, murine models of herpesvirus infection were used to assess the potential of IFNα and IL-12 in combination. In both systemic and zosteriform Herpes Simplex Virus (HSV) infection, IFNα and IL-12 cotherapy improved the survival rate of an otherwise lethal infection to a significantly greater degree than did either therapy alone. Similarly, in Murine Cytomegalovirus (CMV) infection, a combination regime proved efficacious where single cytokine therapy did not influence the outcome of infection. The protection was partly attributed to the induction of IFNγ *in vivo*. Co-administration of IFNα and IL-12 *in vivo* also enhanced the IFNγ producing ability of splenocytes cultured *ex vivo*. IL-12 is an immunomodulator, and IFNα is an inducer of an antiviral state, thus it is likely that the two cytokines in combination exert their antiviral effect by different pathways. This dual mode of action has particular potential application in the treatment of chronic Hepatitis B infection, since IFNα has already shown therapeutic value in treatment of this infection. IFNα mediated reduction in hepatocyte virus replication could be enhanced with timely recruitment of T cell killing of virus infected cells. The mode of action of IL-12 is appropriate for stimulation of effective T cell cytotoxicity. Enhanced clearance of virus from the liver would lessen the liklihood of eventual liver damage resulting from persistent immune activity in this organ.

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Carbocyclic Guanosine Nucleoside BMS-200475 Treatment Inhibits Woodchuck Hepatitis Virus (WHV) Viremia in WHV-Carriers. E.V. Genovesi, L.M. Lamb, I. Medina, J.M. Clark, D.O. Taylor, M. Seifer, S. Innaimo, D.N. Standring, J.E. Sundeen, G.S. Bisacchi, A. Martel, R. Zahler, R.J. Colonno. Bristol-Myers Squibb Company, Wallingford, CT 06492.

BMS-200475 (475), a carbocyclic guanosine nucleoside, inhibited human hepatitis B virus replication *in vitro* in HepG2.2.15 cells with an EC_{50} of 3 nM. 475 was tested for its effect on WHV-viremia in chronically infected WHV-carrier woodchucks in an 84 day therapy protocol. 475 was given orally once a day at either 0.02 or 0.1 mg per kg body weight to respective groups of 6 and 7 animals. Also included was a 4 animal placebo-treatment group. During the period of drug treatment and a subsequent 12 week post-treatment interval animals were bled weekly for serum samples. WHV-viremia was measured as the WHV DNA content in sera using a radiolabled whole-genomic WHV DNA dot-blot hybridization method. By one week of therapy with either 475 dose, WHV DNA was reduced ≥10- to 1000-fold. For all carriers in 475 therapy with 0.1 mg per kg dose, and for 4 of the 6 carriers treated with 475 at 0.02 mg per kg, WHV-viremia was reduced >1000-fold (≤500 pg DNA per ml of serum, the assay detection limit) by the third week of therapy, and remained suppressed for 6 to 8 weeks after therapy cessation. By 8 to 12 weeks after therapy cessation, serum WHV DNA either returned to pretreatment levels or had elevated to detectable levels. In placebo-treated WHV-carriers viremia was unchanged. Thus, 475 showed excellent efficacy in reducing WHV-viremia and should be very effective in the therapy of chronic human hepatitis B virus infections.

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Evaluation of the Antiviral Nucleoside, Lobucavir, in Chronically Woodchuck Hepatitis Virus (WHV) Infected Woodchucks. I. Medina, J.M. Clark, L. Lamb, E.V. Genovesi, D. Taylor, D. Standring, M. Seifer, S. Innaimo and R.J. Colonno. Bristol-Myers Squibb Company, Wallingford, CT 06492.

Lobucavir, BMS-180194, a nucleoside with activity against a variety of herpesviruses also has activity against human hepatitis B (HBV) with an EC_{50} of 2.5 μM as determined in HepG2.2.15 cells. The efficacy of lobucavir was determined in the woodchuck model of chronic hepatitis B infection. Animals chronically-infected with WHV were treated orally with daily doses of 5 to 20 mg/kg per day of lobucavir for 4 to 12 weeks. Animals were bled at weekly intervals and serum levels of WHV DNA determined by a dot blot hybridization technique using a radiolabeled whole genomic WHV DNA probe with a sensitivity of 30 pg per dot. Lobucavir was effective at reducing serum levels of WHV DNA in the woodchuck when administered daily at 10 or 20 mg/kg. Serum levels of WHV DNA were below detectable levels after 1-2 weeks of treatment and returned to pretreatment levels within 1-2 weeks after therapy was stopped. Some animals treated daily with 5 mg/kg of lobucavir showed a decrease in serum levels of WHV DNA to below detectable levels whereas the majority of animals treated with this 5 mg/kg had no significant decrease in WHV DNA. Thus the effective dose of lobucavir in the woodchuck is 10 mg/kg. Lobucavir is effective in reducing serum levels of WHV DNA in WHV chronically-infected woodchucks and should prove of value in the therapy of chronic HBV infection in man.

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Comparative Activation of 2'-Deoxycytidine and Antiviral Oxathiolane Cytosine Nucleosides by Different Mammalian Liver Extracts. Schinazi, R.F.,[1]* Hough, L.,[1] Juodawlkis, A.,[1] Marion, P.,[2] and Tennant, B.[3] Emory University/VA Medical Center, Decatur, GA,[1] Stanford University, CA,[2] and College of Vet. Med., Cornell Univ., Ithaca, NY, USA.[3]

Oxathiolane cytosine nucleosides represent one of the most promising classes of compounds with potent and selective antiviral activity against HIV and hepatitis B virus (HBV). The 2 enantiomers of 2',3'-dideoxy-3'-thiacytidine (BCH-189) and their 5-fluoro analogs (FTC) were previously shown to be good substrates for human 2'-deoxycytidine kinase. The increased clinical interest in these compounds and their use as controls in animal models for HBV led us to study their activation in liver extract obtained from humans, woodchucks, ducks, rats, and mice. We wished to evaluate whether activation was a factor since significant inter-laboratory differences in the therapeutic response of 3TC in chronically infected (WHV) woodchucks have been reported. For these studies, the levels of nucleotide monophosphate formed was determined using a cocktail containing tritium labeled 2'-deoxycytidine and 3TC and DE81 filters which trap the phosphorylated nucleotide, but not the free nucleoside. The levels of 2'-deoxycytidine kinase for the different liver extracts decreased in the following order: mouse > human ≈ woodchuck > duck > rat. For 3TC, the level of activation was 110- (human) to 1,560- (mouse) fold less than for 2'-deoxycytidine. The order of 3TC activation in various liver extracts decreased in the order: human ≈ infected woodchuck (8 mo. old) > mouse ≈ duck ≈ uninfected and infected woodchuck (≥ 12 mo. old) > rat. Younger infected woodchuck livers had mean kinase levels comparable to human livers (1.12 *vs.* 1.24 pmoles/mg/hr). However, for older woodchucks the 3TC phosphorylation levels were between 0.18 and 0.82 pmoles/mg/hr. The results demonstrate that woodchuck and duck liver extracts have high levels of 2'-deoxycytidine kinase and can activate 3TC to its 5'-monophosphate. (Supported by VA and NIH).