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Reduced Replication Efficiency of 3TC-Resistant Human Hepatitis B Virus

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All members of the *hepadnavirus* and *retrovirus* families encode a polymerase with reverse transcriptase activity that is necessary for virus replication. This protein has a catalytic core which contains a highly conserved YMDD motif. Others have shown that human immunodeficiency virus and duck hepatitis B virus with a YVDD change in this motif had reduced sensitivity to 3TC. In addition, 3TC-resistant HIV replicated less efficiently than wild type. To determine the effect this variation has upon human hepatitis B virus (hHBV), we created a transition mutation (adenine to guanine) in the first position of the codon for amino acid 539 (methionine) of the polymerase in a cDNA copy of the pregenomic RNA of hHBV (strain *ayw*) by site specific mutagenesis. This mutation should result in a methionine to valine change at this position. Transfection of Hep G2 human hepatoblastoma cells with wild type or mutant DNA demonstrated that the mutant DNA was less efficient in producing progeny virus than wild type. The efficiency of replication was normalized to the transfection efficiency by co-transfection with marker DNA. Moreover, the resultant virus was less sensitive than the wild type to the antiviral effects of 3TC. These data suggest that a single mutation which causes a methionine to valine change at position 539 of the hHBV polymerase reduces replication efficiency and confers resistance to 3TC.

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EFFECT OF β-ENANTIOMERIC AND RACEMIC NUCLEOSIDE ANALOGUES ON MITOCHONDRIAL FUNCTIONS IN HEPG2 CELLS

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A group of enantiomeric nucleoside analogues with ß-D or ß-L configuration which represent potential candidates for treatment of hepatitis B virus (HBV) infection, was incubated in human hepatoblastoma HepG2 cells at concentrations between 0.1 µM and 10 µM for 4 to 14 days and effects on mitochondrial functions were evaluated. No effect on lactic acid production was detected in cells treated with ß-L-2',3'-dideoxy-3'-thiacytidine (3TC), ß-L-2',3'-dideoxy-5-fluoro-3'-thiacytidine (ß-L-FTC), ß-D-2',3'-dideoxy-5-fluoro-3'-thiacytidine (ß-D-FTC), racemic *cis* 2',3'-dideoxy-5-fluoro-3'-thiacytidine [(±)-FTC], and ß-D-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine (ß -D-D4FC), whereas a slight increase was associated with ß-D-2-hydroxymethyl-5-(2,6-diaminopurin-9-yl)-1,3-dioxolane (ß-D-DAPD) at 10µM. A concentration-dependent increase on lactic acid production was observed in cells exposed to ß-D-2',3'-dideoxy-3'-thiacytidine [(+)-BCH-189], racemic *cis* 2',3'-dideoxy-3'-thiacytidine [(±)-BCH-189], ß-D-2',3'-dideoxy-5-fluorocytidine (ß-D-FddC), ß-L-2',3'-dideoxy-5-fluorocytidine (ß-L-FddC), and ß-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane (ß-D-FDOC), inhibition on mtDNA content was demonstrated to be concentration-dependent with ß-L-D4FC, (+)-BCH-189 and ß-D-FddC had no effect. ß-D-FDOC resulted in a marked inhibition of mtDNA synthesis at 10 µM but not at lower concentrations. Increased cytoplasmic lipid droplets associated with a loss of cristae in mitochondria was detected in cells treated with either ß-D-FDOC or ß-D-FddC. (+)-BCH-189 treatment resulted in loss of cristae in mitochondria. In summary, ß-L-FTC, ß-D-FTC, (±)-FTC, ß-D-DAPD, ß-D-D4FC exhibited a relatively safe profile as 3TC, supporting their further development.

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Pharmacokinetic and Pharmacodynamic Studies of 1-(2-Fluoro-5-methyl-β-L-arabinofuranosyl) uracil (L-FMAU) in the Woodchuck Model of Hepatitis B Virus (HBV) Infection. Tennant B, Jacob J, Graham LA, Peek S, College of Veterinary Medicine, Cornell University, Ithaca, NY; Korba B, Gerin JL, Georgetown University School of Medicine, Rockville, MD; Witcher JW, Boudinot FD, Du J, Chu CK, College of Pharmacy, University of Georgia, Athens, GA, USA.

L-FMAU has significant antiviral activity *in vitro* against HBV and is remarkably less cytotoxic than the D-FMAU isomer. Normal adult woodchucks were given a single 25 mg/kg dose of L-FMAU intravenously. The terminal phase T½ of L-FMAU was 7.2 ± 1.5 hr, total clearance was 0.23 ± 0.07 L/hr/kg, and the steady state volume of distribution was 1.0 ± 0.2 L/kg. The bioavailability of L-FMAU after oral administration of a single 25 mg/kg dose was 25.5 ± 5.3%. L-FMAU was given orally to 4 chronic WHV carrier woodchucks at a daily dose of 10 mg/kg for 12 weeks. There was prompt and significant inhibition of viral replication compared to placebo-treated controls. Within 2 weeks, the serum WHV DNA of drug-treated woodchucks decreased more than 1,000-fold and thereafter during treatment was undetectable by conventional blot hybridization. The initial T½ of plasma WHV DNA in drug-treated woodchucks was 17 ± 1 hr. At the end of treatment, hepatic WHV DNA replicative intermediates were decreased more than 10-fold over pretreatment and control levels. Based on immunohistochemical analysis, hepatic expression of WHcAg also was diminished after 12 weeks of treatment. Variations in body weight of L-FMAU-treated woodchucks were similar to those of controls, and no other clinical evidence of drug-related toxicity was observed. These results suggest that L-FMAU may be valuable for treatment of HBV infection and that the woodchuck model will be useful in assessment of the long-term effects of treatment on the course of persistent hepadnaviral infection.

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Effectiveness of Combination Therapies with 3TC, Famciclovir, and Alpha Interferon against Woodchuck Hepatitis Virus Replication in Chronically-infected Woodchucks: Model for Potential Anti-HBV Treatments. BE Korba[1], B Baldwin[2], P Cote[1], R. Schinazi[3], D Gangemi[4], JL Gerin[1], BC Tennant[2].1- Georgetown Univ., DMVI, Rockville, MD; 2-Coll. Vet. Med., Cornell Univ., Ithaca, NY; 3-Emory Univ., Atlanta, GA; 4-Clemson Univ., Clemson, SC.

Lamivudine (3TC), famciclovir (Famvir™, penciclovir), and alpha interferon have been shown to be highly effective inhibitors of hepadnaviral replication in cell culture, experimental animal models, and in human clinical trials. However, all of these agents fail to permanently eliminate virus replication in the vast majority of patients, even after prolonged administration. A rapid rebound in virus production usually occurs once treatment is discontinued. Combination therapies with two or more of these drugs may be able to more effectively suppress viral replication. We have utilized the highly predictive 2.2.15 cell and WHV/woodchuck systems to model antiviral therapies with these agents. Culture studies using the 2.2.15 cell line have demonstrated that combination treatments with 3TC and either interferon or penciclovir reduced the 90% effective concentrations of these agents 10 to 30-fold (BE Korba, Antiviral Res. *29:49*). Monotherapy with interferon, 3TC, or famciclovir suppressed WHV viremia in chronically infected woodchucks approximately 10 to 300-fold after 12 to 24 weeks of treatment at doses which are the approximate equivalent (based on metabolic body size ,$K^{3/4}$) to those used in humans. Combination treatments with 3TC and either interferon or famciclovir reduced WHV viremia by the end of the treatment periods significantly more than the corresponding monotherapies and significantly more than the levels predicted for additive antiviral effects. However, following the withdrawal of treatment, essentially no differences were observed in the rebound of WHV viremia between the combination and monotherapies. These studies demonstrate the potential utility of combination therapies with these clinically relevant antiviral agents and may help to establish parameters for the use of multiple drug treatments against chronic HBV infection.