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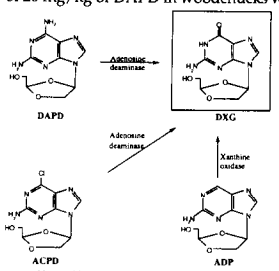
Anti-HBV and Anti-HIV Activities of Dioxolane-Purine Nucleosides. R.F. Schinazi,* F.D. Boudinot,† K.K. Manouilov,† J.W. Mellors,‡ A. McMillan,* S. Schlueter-Wirtz,* R. Lloyd,* jr., B.E. Korba,¥ B. Tennant,** and C.K. Chu.† *Veterans Affairs/Emory Univ. School of Medicine, Atlanta, GA 30033, †Univ. of Georgia College of Pharmacy, Athens, GA 30602, ¥Georgetown Univ. Mol. Virology and Immunol., Rockville, MD 20852, **Cornell Univ. College of Veterinary Med., Ithaca, NY 14853, and ‡Veterans Affairs/Dept. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261.

During our efforts to discover safe and effective anti-HBV and anti-HIV nucleosides, a number of enantiomerically pure dioxolane nucleosides were synthesized. From these studies, several promising dioxolane nucleosides had dual activity, including DXG, DAPD, ADP, and ACPD. *In vitro* and *in vivo* pharmacokinetic studies demonstrated that DAPD, ADP and ACPD served as prodrugs of DXG in rhesus monkeys and woodchucks. Based on the *in vitro* antiviral data, solubility in water, as well as availability of the compound, DAPD was further pursued as a potential antiviral agent. The pharmacokinetics of DAPD and DXG following i.v. and p.o. administration of 20 mg/kg of DAPD in woodchucks was investigated. DAPD was extensively converted to DXG. The half-lives of DAPD and DXG after administration of DAPD were 6.7 h and 17.5 h, respectively. The oral bioavailability of DAPD was only 6.6%. However, considering the conversion of DAPD to DXG, the oral bioavailability of DAPD was 40.1%. A 3 month efficacy study in woodchucks infected with WHV indicated that DAPD was equally effective as 3TC at the same dose and schedule (1 mg/kg, bid). Selection of drug-resistant HIV-1 to DXG in both MT-2 and human PBM cells resulted in resistant viruses. DNA sequence analyses of the RT gene amplified from these viruses consistently identified a single mutation at codon 65 from K (AAA) to R (AGA). This suggests that DXG and its prodrugs can be used in combination with the oxathiolane nucleosides 3TC and FTC, as well as AZT for the therapy of HIV. It is anticipated that these new purine dioxalanes will also prove useful for HBV infections since they are not co-resistant with 3TC (Supported by NIH grants AI-32351, AI-33655, NO1-AI-35164, and Veterans Affairs).



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INFLUENCE OF HEPATITIS B VIRUS GENOTYPE ON INTERFERON RESPONSE IN HBe ANTIGEN NEGATIVE CHRONIC HEPATITIS B

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The clinical importance of hepatitis B virus (HBV) genome variability has been recently uncovered. One example is the occurence of Hepatitis B virus pre-core mutants which arise during seroconversion from HBeAg to anti-HBe and are thought to be selected by immune pressure. A survey of HBV pre-core mutants and viral genotypes was conducted in 35 HBeAg negative patients during interferon-α therapy to better understand viral pathogenesis in this form of chronic hepatitis B. Seventeen patients responded to interferon therapy as assessed by the sustained normalization of serum ALT levels and the significant decrease of viremia levels determined by bDNA assay (Chiron). The response rate to interferon was independent of both initial serum viral DNA levels and interferon doses. During interferon therapy, a significant decrease of M0 (wild type pre-core sequence at pos. 1887-1908), M1 (TGG->TAG at pos. 1896) or M2 (TGG -> TAG at pos. 1896, and GGC -> GAC at pos. 1899) positive viral genomes was found in 48 % , 42 % and 33% of patients respectively. This was confirmed by a follow-up analysis of pre-core mutants and wild type strains by a quantitative PCR assay. A higher response rate to interferon therapy was observed in patients infected with HBV genotype A (70%) or M0 positive strains (75%) as compared to patients infected with genotype D/E (40%) or M1/M2 positive strains (44%) ($p<0.05$). All 5 patients who were cured from HBV infection were initially infected by HBV genotype A and M0 positive strain. Our data suggest that characterization of HBV genome variability prior to interferon therapy may help to predict antiviral response in HBe Ag negative patients.

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2'FLUORO 5 METHYLβL-ARABINOFURANOSYLURACIL, A NOVEL L-NUCLEOSIDE ANALOG, INHIBITS DUCK HEPATITIS B VIRUS REPLICATION IN PRIMARY HEPATOCYTES AND IN VIVO

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β-L-nucleoside analogs represent a new class of potent antivirals which provide new hope in the therapy of chronic hepatitis B virus (HBV) infections. Among these, a novel anti-HBV compound, 2'-Fluoro-5-methyl-β-L-arabinosyluracil (L-FMAU), was found to be a potent inhibitor of HBV replication in a stably transfected hepatoma cell line (F2215) and to have a low in vitro cytotoxicity. We have therefore evaluated its anti-HBV activity in the duck hepatitis B virus (DHBV) model. Using a cell free system for the expression of an enzymatically active DHBV polymerase, the triphosphate form of β-L-FMAU was shown to inhibit viral minus strand DNA synthesis, ie reverse transcription. In primary duck hepatocyte culture, β-L-FMAU therapy for 9 days showed a potent inhibitory effect (IC50 = 0.1 μM) on DHBV DNA synthesis which was concentration dependent and similar to that of 2',3'-dideoxy-β-L-5-Fluorocytidine (β-L-FddC). The combination of β-L-FddC and β-L-FMAU was not synergistic in terms of antiviral effect. The oral administration of β-L-FMAU (40mg/kg/d, 5 days) in 4 experimentally infected ducklings induced a 72% inhibition of viremia levels as compared to the control group (5 animals). A pulse therapy by β-L-FMAU before inoculation (5 animals) could delay the onset of viremia by one day and decreased the peak of viremia by 65 % as compared to the control group. These data show that β-L-FMAU inhibits hepadnavirus reverse transcription and is a strong inhibitor of viral replication both in vitro and in vivo. Further experiments are underway to study its mechanism of action in detail.

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SERUM HCV RNA LEVELS USING BY THE BRANCHED DNA AMPLIFICATION ASSAY IN CHILDREN WITH CHRONIC HEPATITIS C

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Serum HCV RNA levels, measured by PCR methods are useful clinically. However, these methods are expensive and require special skills. A new approach, branched DNA (bDNA) signal amplification, has lately developed. We evaluated the effect of IFN and investigated the bDNA assay that may predict the outcome in 27 children (18 boys, age range 5-19 yr) with chronic hepatitic C. Their underlying diseases were divided into malignancies (Group A, n=20) and non-malignancies (Group B, n=7). These 27 children received 0.1 MIU/Kg of IFN-alfa daily for 2 weeks and then 3 times a week for additional 22 weeks. They were followed for 18 months or longer. A complete response (CR) was obtained in 12 (44%, Group A:30%, Group B:85%). Serum levels of HCV RNA assayed by multicyclic RT-PCR were closely correlated with those by bDNA ($r=0.52$). There were differences in bDNA titers among underlying diseases ($p<0.05$), liver histologic activity ($p<0.05$) and HCV genotype ($p<0.1$). 10 of the 19 children (53%) whose serum bDNA titers were <1Meq/ml had CR, while 2 of the 8 (25%) whose bDNA were ≥ Meq /ml had CR. A bDNA assay, which is easy to examine and not expensive, is useful clinically.