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Antiviral Strategies in Chronic Hepatitis B Virus Infection: Inhibition of Duck Hepatitis B Virus Replication *In Vitro* by Pyrimidine Analogs.

S.A. Locarnini, T. Shaw and D.S.Bowden. Hepatitis Research Unit, Macfarlane Burnet Centre for Medical Research and Virology Laboratories, Fairfield Hospital, Fairfield, Victoria, Australia.

We have been attempting to develop antiviral strategies which will be effective against chronic human HBV using duck hepatitis B virus (DHBV) as a model. Potential antiviral agents are initially screened for ability to inhibit replication of DHBV in cultures of congenitally infected primary duck hepatocytes (PDH). The validity of this approach depends not only on known similarities between Hepadnaviral replication strategies but on assumed metabolic similarities between duck and human cells. Cultured PDH are quiescent, and, like their human counterparts, lack enzymatic mechanisms for pyrimidine (deoxy)nucleoside salvage. Furthermore, both duck and human hepatocytes rapidly catabolise exogenously supplied pyrimidine nucleosides. Despite these characteristics PDH support replication of DHBV, which requires a continuous balanced supply of deoxynucleotide precursors. How do quiescent (duck and human) hepatocytes sustain the pyrimidine requirement for (D)HBV replication? We investigated this problem by screening pyrimidine analogs for anti-DHBV activity in vitro. In general, pyrimidine nucleoside analogs which could be catabolised deoxycytidine deaminase and/or pyrimidine nucleoside phosphorylase(s) in PDH to yeild uracil analogs were antivirally active whereas those which were not recognised as substrates (including 6-substituted analogs and those with modifications in the sugar moiety) were inactive. Free bases corresponding to the first category were also active. These data imply that PDH possess phosphotransferase(s) which salvage pyrimidine bases and suggest that interference with pyrimidine nucleotide synthesis at this or earlier stages may be targets for antiviral (D)HBV action.

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INHIBITORY EFFECTS OF PHOSPHONATE NUCLEOTIDE ANALOGUES ON HUMAN HEPATITIS B VIRUS DNA SYNTHESIS

T.Yokota, K.Konno,S.Shigeta,A.Holy[1],J.Balzarini[2] and E.De Clercq[2]
Department of Microbiology, Fukushima Medical College, Fukushima 960-12,Japan, [1]Institute of Organic Chemistry and Biochemistry, 166 10, Praha, Czechoslovakia and [2] Rega Institute for Medical Research, Katholieke Universiteit Leuven,B-3000,Leuven, Belgium

Acyclic phosphonate nucleotide analogues were examined for their inhibitory effects on human hepatitis b virus (HBV) DNA synthesis. The assay system was based on the use of a human hepatoblastoma cell line(HB611) that continuously synthesizes HBV DNA. Out of 56 tested compounds the following compounds were found to inhibit HBV DNA synthesis at concentrations that were significantly lower than their minimum cytotoxic concentrations: 9-(2-phosphonylmethoxyethyl)adenine(PMEA),9-(2-phosphonylmethoxy-ethy)-2, 6-diaminopurine(PMEDAP), (S)-9-(3-hydroxy-2-phosphonyl-methoxypropyl)adenine(HPMPA), 9-(3-fluoro-2-phosphonylmethoxy-propyl)adenine(FPMPA),9-(3-isopropoxy-2-phosphonylmethoxypropyl) adenine(IPPMPA) and 9-(RS)-(2-phosphonylmethoxypropyl)adenine[(RS)PMPA]. Acyclic phosphonate pyrimidine nucleotide analogues tested did not show high selectivity as anti-HBV agent. Diphosphoryl derivatives of PMEA, PMEDAP, HPMPA and FPMPA were inhibitory to a endogenous HBV DNA polymerase.