169

The Inhibition of Flavivirus RNA Synthesis by the Heteropolyanion HPA-23 and Related Compounds in the In Vitro RNA-Dependent RNA Polymerase Assay

A.Bartholomeusz (1 & 2), P.J.Wright (1), E.Tomlinson (1), S.Locarnini (2), C.Birch (2), H.Weigold (3), S.Marcuccio (3) & G.Holan (3).
(1) Dept. of Microbiology, Monash Univ., Clayton, AUSTRALIA
(2) Virology Dept. Fairfield Hosp. Fairfield, AUSTRALIA
(3) Division of Chemicals and Polymers CSIRO, AUSTRALIA

The family Flaviviridae includes the genera flavivirus, pestivirus and hepatitis C virus. These viruses copy their genomes via a virus encoded RNA dependent RNA polymerase (RDRP). An RDRP assay has been utilised to investigate the effects of several antiviral agents against flavivirus RNA synthesis. In this assay the radiolabel [α-^{32}P]GTP is incorporated into all three forms of intracellular viral RNA; the single-stranded genomic length RNA, the double-stranded replicative form (RF) and the partially single stranded replicative intermediate RNA. The RF RNA may have an important role in flavivirus RNA replication. It has been proposed that the RF RNA acts as a template for the preferential synthesis of genomic RNA. Thus, the RF RNA could represent an important target for the development of antiviral agents. Several classes of known polymerase inhibitors were tested in the RDRP assay including polymerase binding compounds, nucleoside analogues, template binding compounds and a substrate analogue. We found the synthesis of RF RNA was greatly reduced in the presence of 5 μM HPA-23, a polymerase binding compound. The antiviral activity of related compounds will be discussed. The RDRP assay should prove useful in investigating compounds which are uniquely targeted to viral RNA synthesis.

170

Inhibition of duck hepatitis B virus replication by *N*-(phosphonoacetyl)-L-aspartate (PALA; USNUS-08-0) *in vitro*.

T. Shaw, S.A. Locarnini, and H.A. Blough#. Macfarlane Burnet Centre for Medical Research and Victorian Infectious Diseases Reference Laboratory, Fairfield Hospital, Victoria, 3078, Australian and #US Bioscience, West Conshohocken, PA 19428, USA.

Work from a number of laboratories including our own indicates that pyrimidine nucleoside analogues are generally less effective antihepadnaviral agents than their purine counterparts. Susceptibility to catabolism and competition from endogenous pyrimidines synthesised *de novo* are amongst the reasons for the relatively poor efficacy of the former group both *in vitro* and *in vivo*. A rational approach to chemotherapy against hepadnaviruses might therefore include inhibition of *de novo* pyrimidine synthesis with or without interference with other pathways. Here we report that treatment with clinically achievable, non-cytotoxic concentrations of PALA (USNUS-08-0), a stable tight-binding enzyme inhibitor which blocks the second step of *de novo* pyrimidine biosynthesis, causing a dramatic dose-dependent inhibition of duck hepatitis B virus replication and virus-specific protein synthesis in congenitally infected primary duck hepatocytes *in vitro*. Viral DNA replication and viral core, pre-S1 and pre-S2 antigen synthesis were inhibited by 50-90% at drug concentrations in the range 10-100μM, varying between different primary hepatocyte cultures. Our data suggest that PALA may be a useful antihepadnaviral agent *in vivo*, either alone or in combination with other agents.