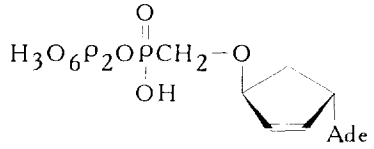
249

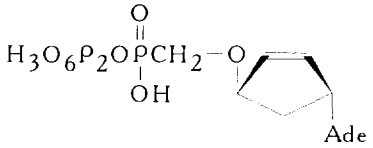
Highly Selective Inhibitors of Terminal Deoxynucleotidyl Transferase in Cell Free Systems

A.A.Krayevsky, N.B.Dyatkina, D.G.Semizarov, M. von Janta-Lipinski*
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov St., Moscow 117984, Russia, *Max-Delbruck Centre of Molecular Medicine, Robert-Rossle Street 10, Berlin-Buch, D-13125 Germany

Terminal deoxynucleotidyl transferase (TDT) is a template-independent DNA polymerase expressed in pre-lymphocytes (in thymus and marrow) and in their neoplastic counterparts - lymphoblastic leukaemic cells. The biological function of TDT has not been elucidated yet, and partly due to the lack of specific inhibitors of this enzyme. We evaluated compounds I and II in cell free system wit TDT and showed them to be very specific terminating substrates of calf thymus TDT. Both D-α (I) and L-β (II) compounds had the



I



II

trans-like configuration; they were not recognized by several human template-dependent DNA polymerases and retroviral reverse transcriptases. Meanwhile, their *cis*-like counterparts (D-β and L-α) terminated DNA synthesis catalyzed by some template-dependent DNA polymerases and TDT. The kinetic constants for I and II in the primer extention reaction catalyzed by the TDT are shown to be 2-3 fold higer as compared with those for corresponding *cis*-like isomers. The data obtained demonstrate the possibility of finding high specific TDT inhibitors among *trans*-like dNTP for cell-free media. Parent nucleosides and nucleotides could be studied in cell cultures.

250

Effects of inhibitors of intracellular transport on the replication of Junin virus. E.B. Damonte, N.A. Candurra. Laboratorio de Virología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires Argentina.

The processing and transport of viral membrane glycoproteins represent a target for antiviral agents. In the present study, the activity of diverse inhibitors of intracellular transport has been assayed against Junin virus (JV), agent of Argentine Hemorrhagic Fever. The tested compounds were carbonyl cyanide m-chlorophenylhydrazone (CCCP), which blocks transport from the endoplasmic reticulum (ER); brefeldin A (BFA), a fungal metabolite which blocks anterograde transport from the ER whereas retrograde transport continues, and caffeine, a substance with many pharmacological actions which also affects the exocytic pathway. All the compounds were found to be active against the multiplication of JV, strain IV4454, on Vero cells in a virus yield reduction assay. Virus production was inhibited in a dose dependent form at the range of concentrations 0.1-10 ug/ml for BFA, 10-50 ug/ml for CCCP, and 2-20 mM for caffeine, whereas cell viability was not affected. Both extracellular and cell-associated virus yields were significantly reduced. A similar level of inhibition was achieved if BFA was added immediately after virus adsorption or so late as 18 h post-infection, indicating that a late stage on the viral cycle was blocked. Immunofluorescence localization studies with specific monoclonal antibodies and analysis of the proteolytical cleavage of the GPC precursor protein suggested that in the presence of the compounds transport to the cell surface of the mature JV envelope glycoprotein GP38 was arrested. Thus, this protein transport inhibition blocked the production of JV infectious virions.