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Interaction of the Carbocyclic Analog of 2'-deoxyguanosine (CdG) with Herpes Simplex Virus Type I (HSV) Thymidine Kinase (TK). P. W. Allan, W. B. Parker, and L. L. Bennett, Jr., Southern Research Institute, Birmingham, AL 35255 USA.

CdG is of interest because of its activity against herpes and hepatitis B viruses. We have shown that D-CdG is phosphorylated extensively in HSV-infected cells and very poorly in uninfected cells (Biochem. Pharmacol. 40: 1515, 1990). The D-enantiomer of CdG was 500-fold more potent as an inhibitor of HSV replication than the L-enantiomer. Consistent with these observations no phosphorylation of unlabeled L-CdG was detected in HSV-infected cells. We have now examined D- and L-CdG as substrates for the HSV-TK partially purified from TK-minus HeLa cells infected with HSV (Strain S-148). [8-^{3}H]D-CdG was a substrate for the HSV-TK; K_m, 14 μM; V_{max}, 1.4 nmoles/hr/mg of protein. Substrate inhibition was observed with D-CdG. The K_m and V_{max} for thymidine was 0.4 μM and 0.76 nmoles/hr/mg of protein, respectively. Both D- and L-CdG were equally potent as inhibitors of the phosphorylation of thymidine by the enzyme; IC_{50} of approximately 20 μM at a thymidine concentration of 1 μM. Thus L-CdG appears to be an inhibitor but not a substrate of this enzyme; inhibition of this enzyme could be responsible for the antiviral activity of this compound. Substrate kinetics for L-CdG with HSV-TK are under investigation. Also under study is the phosphorylation of CdG in uninfected cells. Stimulation of the phosphorylation of CdG by mycophenolic acid in whole cells suggests that 5'-nucleotidase is responsible for this activity. This work was supported by NCI grant number CA34200.

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Interaction of 5-methoxymethyldeoxyuridine triphosphate with DNA polymerases: effects of the 5-substituent and comparison with the deoxycytidine derivative. Stuart, A.L., Gupta, V.S. and Aduma, P.J. Dept. of Vet. Physiol. Sci., Univ. of Saskatchewan, Saskatoon, Sk., Canada.

Methoxymethyldeoxyuridine (MMdUrd) is a selective antiherpes agent that is dependent upon initial phosphorylation by Herpes simplex virus-induced deoxythymidine kinase. In order to determine its mechanism of action, MMdUrd was converted to the 5'-triphosphate (MMdUTP) and the nature of interaction of MMdUTP and dTTP with DNA polymerase of *E. coli*, HSV-1 and human α was investigated. The order of utilization of deoxythymidine analogs by bacterial and HSV-1 DNA polymerases for DNA synthesis was: dTTP > MMdUTP. In contrast, 5-methoxymethyldeoxycytidinetriphosphate (MMdCTP) was a better substrate for HSV DNA polymerase compared to dCTP. MMdUTP is a competitive inhibitor of HSV-1 DNA polymerase with respect to dTTP incorporation (K_i=2.9x10^{-6} M). The IC_{50} values of MMdUTP for both HSV and Human DNA polymerases were 4.5x10^{-6} M. These data suggest that the selective activity of MMdUrd is due to its preferential phosporylation by viral kinase and not at the DNA polymerase level. These results may also account for the difference in anti-HSV activity between MMdUrd and its deoxycytidine analog.

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