95

Mechanism of Selective Inhibition of Herpes Simplex Virus Replication by Deoxycytidine Analogs: Interaction of 5-Methoxymethyl-2'-deoxycytidine-5'-triphosphate with DNA Polymerases. V.S. Gupta, P.J. Aduma, H.S. Allaudeen, A.L. Stuart and G. Tourigny. Dept. of Veterinary Physiological Sciences and Chemistry, University of Saskatchewan, Saskatoon, SK, S7N OWO, Canada.

5-Methoxymethyl-2'-deoxycytidine (MMdCyd) is a selective antiherpes agent that is dependent on initial activation by Herpes simplex virus (HSV)-induced deoxythymidine/deoxycytidine kinase. 5-Methoxymethyl-2'-deoxycytidine triphosphate (MMdCTP) was synthesized. The nature of interaction of MMdCTP and dCTP with DNA polymerase of E. coli; HSV-1 and human α was determined using specific and optimized assay conditions for each enzyme. MMdCTP was a better substrate for HSV-1 DNA polymerase as compared to dCTP. At 10 μM nucleotide concentration, MMdCTP utilization was 130% compared to that for an equimoler concentration of dCTP. Under similar conditions, human DNA polymerase α was less efficient in utilizing MMdCTP as an alternate substrate. E. coli DNA polymerase 1 preferentially utilized dCTP. The IC_{50} values of MMdCTP were 8×10^{-7} M and 29×10^{-7} M for HSV-1 and human α DNA polymerase respectively. MMdCTP is a competitive inhibitor of HSV-1 DNA polymerase with respect to dCTP incorporation (Ki = 3.8×10^{-7} M). Preferential utilization of MMdCTP and its eventual incorporation accounts for the antiviral action of MMdCyd which appears distal to the polymerization reaction. The low capacity of human DNA polymerase α to utilize MMdCTP is further evidence in support of the low cytoxicity of MMdCyd.

Supported by the MRC and CIDA.

96

Resistance Artifact From an Automated Assay Observed in Viral Isolates from Patients with Frequent Genital Herpes Simplex Virus (HSV) Infections Serially Treated with Topical 3% Edoxudine (EDU) Cream. SL Sacks. University of British Columbia, Vancouver, BC, Canada.

The clinical significance of antiviral resistance during HSV infections has been difficult to demonstrate, since patients may have persistent lesions caused by susceptible virus or recover from infections caused by resistant isolates. Prolonged or intermittent exposures to antivirals have led to resistance, predominantly in the compromised host. Recently, we studied serial antiviral susceptibilities to EDU and acyclovir (ACV) in normal hosts with frequently recurrent HSV-2 infections, repeatedly exposed to topical 3% EDU cream. Susceptibilities were assayed in Vero cells, using cytopathic effect (CPE) reduction with neutral red uptake in 96 well plates at 4 HSV inoculum levels, and computerized analysis of ID_{50} based on absorbance (570/410). Isolates with ID_{50}'s suggestive of resistance were retested by manual plaque reductions in Vero cells. Clinical isolates from separate treated episodes in 34 patients displayed a median ID_{50} of 4.7 ± 5.6 µg/ml when tested by cpe reduction at 1 DU_0 (complete CPE within 48h of inoculation). Three of 113 isolates so tested showed median ID_{50} values in excess of 50 µg/ml and 2 others $\geq$ 30 µg/ml. By contrast, simultaneous CPE reductions against ACV showed a median ID_{50} 0.74 ± 1.4 µg/ml, with no suggestion of resistance. Lack of resistance against EDU was then confirmed by plaque assay conducted in the 5 resistant-appearing isolates. ID_{50}'s against EDU in this "resistant" population yielded a mean ID_{50} of 1.17 µg/ml against EDU by plaque reduction, well within the range of susceptibility. Despite the difference in median values of ID_{50} between ACV and EDU, ratios of ID_{50} measured at differing inocula were statistically similar for each of these 2 agents. Accordingly, inoculum effects were not responsible for the disparity of "apparent resistance" observed between ACV and EDU using the CPE reduction assay. Furthermore, the appearance of apparent resistance could not be correlated to clinical or therapeutic status. Thus, repeated exposure to topical 3% EDU did not predispose to the development of either apparent resistance or true resistance. These results confirm the utility of the automated CPE reduction assay for studies of ACV resistance. However, the validity of an automated assay should be separately determined for each antiviral agent. Furthermore, true resistance should be confirmed by a second assay.