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Comparative Efficacy and Selectivity of Some Nucleoside Analogs Against Epstein-Barr Virus

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The effects of (2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluridine (FMAU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine (FIAU), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU), and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG or BW B759U) on the replication of Epstein-Barr virus (EBV) in vitro were evaluated and compared with that of acyclovir (ACV). The relative potencies of these drugs, on the basis of anti-EBV activity, were: FIAC = FIAU > FMAU > DHPG > BVdU > ACV; on the basis of the therapeutic index they were: BVdU > DHPG > FIAC > ACV > FIAU > FMAU. Differential inhibition of EBV-associated polypeptides by these drugs was observed.

We recently reported that several nucleoside analogs, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU), and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG or BW B759U), are potent inhibitors of Epstein-Barr virus (EBV) replication in vitro (14, 16). These four drugs have prolonged effects in suppressing viral replication, even after the drugs are removed from persistently infected cell cultures (14, 16). In the present studies we have extended our previous findings and further characterized the efficacy of each drug in parallel with acyclovir (ACV) in terms of inhibition of replication of EBV in human lymphoblastoid cell lines.

Exponentially growing P3HR-1 cells were fed (at 3-day intervals) with fresh medium containing different concentrations of drugs (0, 0.01, 0.1, 1, 10, and 100 μM) and incubated for 14 days (16). At the end of drug treatment, cells were harvested, and the number of EBV genome copies per cell was determined by cRNA-DNA hybridization with an EBV-specific cRNA probe as detailed elsewhere (13, 15).

A dose-dependent inhibitory effect was observed for each drug tested. The number of EBV genome copies per cell was plotted against drug concentrations on a semilogarithmic scale. Taking as the zero point the residual level of 30 genome copies per cell (16), from the plots we determined the viral 50 and 90% effective doses (ED_{50} and ED_{90}) of each drug. The same graphical method was used to determine the cell growth 50% inhibitory doses (ID_{50}). The ID_{50}s for BVdU, FIAC, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine (FIAU), and FMAU were, respectively, 390, 5, 1, and 1 μM. On the basis of these data, we calculated the therapeutic index (ID_{50}/ED_{50}) for each drug (Table 1). For comparative purposes, data on ACV and DHPG which were published previously (16) are included.

The relative potencies of these drugs on the basis of anti-EBV activities were: FIAC = FIAU > FMAU > DHPG > BVdU > ACV; on the basis of the therapeutic index they were: BVdU > DHPG > FIAC > ACV > FIAU > FMAU (Table 1).

We compared the effects of BVdU, FIAC, FMAU, ACV, and DHPG on the synthesis of EBV-associated polypeptides by polyacrylamide gel electrophoresis (12). Figure 1 shows the results of a fluorogram (1) made by exposing an electropherogram of ^{35}S-labeled polypeptides synthesized in superinfected Raji cells in the presence and absence of drugs. Superinfection of Raji cells (lane S), compared with mock-infected cells (lane M), resulted in the synthesis of at least seven new polypeptides with molecular weights of 145,000, 140,000, 135,000, 110,000, 85,000, 55,000, and 32,000, which were detected 24 h postinfection in a continuous labeling experiment. The polypeptides with molecular weights of 140,000 and 145,000 were partially inhibited by ACV, FIAC, and FIAU, in contrast to being markedly reduced by BVdU, FMAU, and DHPG. Differential inhibition of the 110,000-molecular-weight polypeptide by these drugs was observed. In addition, synthesis of the 85,000-molecular-weight polypeptide was markedly reduced by DHPG and FIAU, but it was not significantly reduced by FMAU, FIAC, BVdU, or ACV.

The studies presented here clearly demonstrate that DHPG, BVdU, FIAC, FMAU, and FIAU are potent inhibitors of EBV replication in culture. As with ACV, these drugs effectively inhibit linear forms of EBV DNA, which are replicated by virus-specific DNA polymerase, but have no effect on the EBV plasmids, which are presumed to be replicated by host enzyme.

Studies with ACV and BVdU have clearly indicated that preferential phosphorylation of these compounds by the virus-specific thymidine kinase is a prerequisite for their selective activities against herpes simplex virus type 1 (7, 11) and herpes zoster virus (3). Although there is some evidence

TABLE 1. Inhibitory action of nucleoside analogs

Drug	ED_{50} (μM)	ID_{50} (μM)	Therapeutic index (ID_{50}/ED_{50})
ACV[a]	0.3	250	833
DHPG[a]	0.05	200	4,000
BVdU	0.06	390	6,500
FIAC	0.005	5	1,000
FIAU	0.005	1	200
FMAU	0.0065	1	154

[a] Data previously published (16).

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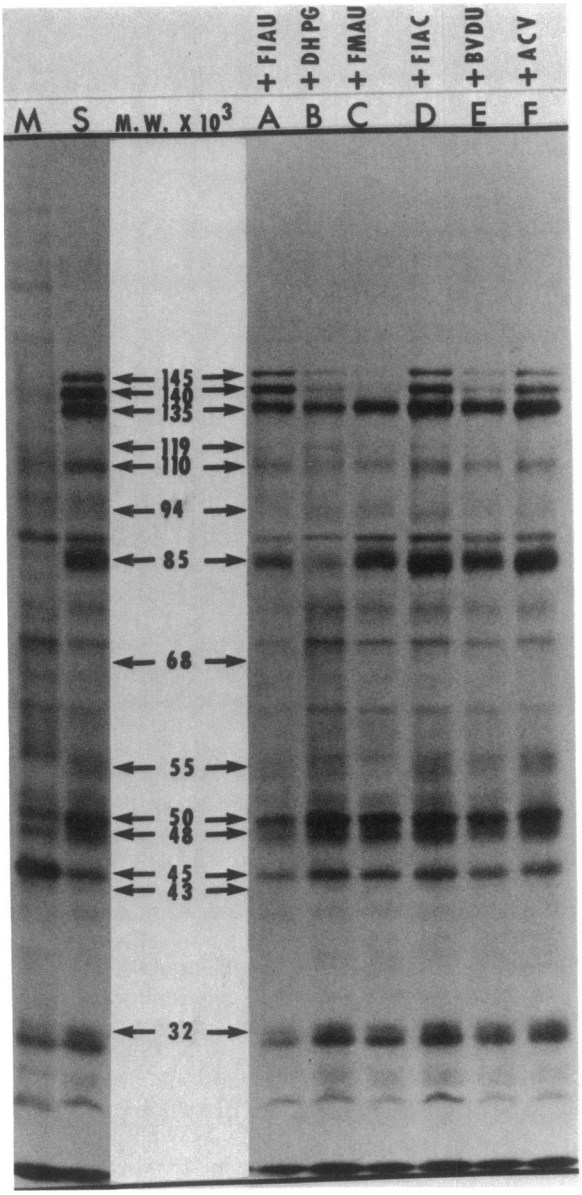

FIG. 1. Differential effects of nucleoside analogs on synthesis of EBV-associated polypeptides. Lanes M and S were polypeptides synthesized in mock-infected and superinfected Raji cells, respectively. Lanes A through F were polypeptides synthesized in superinfected Raji cells in the presence of the drug specified at the top of each lane. Concentrations of the drugs used were 10 times their ED_{90}s, i.e., 40 μM BVdU, 100 μM ACV, 30 μM DHPG, and 1 μM FIAC, FMAU, and FIAU. M.W., Molecular weight.

for and against the existence of an EBV-specific thymidine kinase (2, 5, 6, 17, 18), there has been no unequivocal demonstration of a novel enzyme activity induced by EBV that might represent a virus-encoded thymidine kinase. However, we have found that both DHPG and ACV were preferentially phosphorylated in superinfected Raji cells, in contrast to the low level of phosphorylation in mock-infected Raji cells (J.-C. Lin, D. J. Nelson, C. U. Lambe, E. I. Choi, and J. S. Pagano, *in* H. E. Kaufman and K. Reisaku, ed., *Pharmacological and Clinical Approaches to Herpesvirus and Virus Chemotherapy*, in press). EBV infection produced a rapid and marked increase in DHPG phosphorylating activity, and this activity was 100-fold higher than that of ACV (Lin et al., in press). The larger quantity of DHPG triphosphate than of ACV triphosphate formed in superinfected Raji cells may facilitate the incorporation of DHPG into the internucleotide chain of EBV DNA, as reported in herpes simplex virus-infected cells (4). Whether the prolonged anti-EBV activities of DHPG, FIAC, FMAU, and BVdU previously observed (14, 16) are related to the accumulation of a stable pool of drug triphosphates remains to be determined. It also remains to be established whether the inhibition of EBV replication by these drugs follows a mechanism similar to that reported in herpes simplex virus (8).

The differential effects of the drugs on EBV-associated polypeptides are consistent with their antiviral activities. These findings are similar to our previous work on differential effects of DNA inhibitors on viral polypeptide synthesis (9, 16). As is the case for most antiviral drugs, the effects of these compounds on viral polypeptides are most likely due to inhibition of EBV DNA synthesis. The differential reduction in EBV-induced polypeptide synthesis, although a secondary effect of the drugs, could play a role in the inhibition of virus replication, as suggested in herpes simplex virus systems (10, 19).

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