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Anti-Human Immunodeficiency Virus Agent 3′-Azido-3′-Deoxythymidine Inhibits Replication of Epstein-Barr Virus

JUNG-CHUNG LIN,[1,2]* ZHEN-XI ZHANG,[1] M. CAROLYN SMITH,[1] KAREN BIRON,[3] AND JOSEPH S. PAGANO[1,4,5]

Lineberger Cancer Research Center[1] and Departments of Biochemistry and Nutrition,[2] Medicine,[4] and Microbiology,[5] School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, and The Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709[3]

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We show that the anti-human immunodeficiency virus agent, 3′-azido-3′-deoxythymidine (AZT), which suppresses infectivity and cytopathic effects of human immunodeficiency virus, also effectively inhibits Epstein-Barr virus (EBV) DNA replication. However, AZT has no effect on four other human herpesviruses: cytomegalovirus, varicella-zoster virus, and herpes simplex virus types 1 and 2. The combination of acyclovir and AZT, while it is not synergistic, has an additive effect against EBV replication. AZT may prove to be a useful drug for treatment of coinfections with human immunodeficiency virus and EBV.

In recent years, we have shown that several nucleoside analogs selectively inhibit the replication of Epstein-Barr virus (EBV) (14–17). One drug has been tested in trials in patients with infectious mononucleosis (6, 21; C. M. Van der Horst, J. Joncas, G. Ahronheim, G. Stein, M. Gurwith, G. Fleisher, J. L. Sullivan, J. Sixbey, C. Sumaya, R. Schooley, S. Roland Sweezy, and J. S. Pagano, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 318, 1986). In searching for other antiherpetic agents, we discovered that 3′-azido-3′-deoxythymidine (AZT), which is active against human immunodeficiency virus (HIV) both in vitro (20) and in vivo (26), strongly inhibits EBV replication. Inhibition was surprisingly selective in that the drug showed no detectable effect on replication of the other human herpesviruses, cytomegalovirus (HCMV), varicella-zoster virus (VZV), and herpes simplex virus types 1 and 2 (HSV-1 and HSV-2).

To test the effect of AZT on EBV DNA replication, we used P3HR-1 (LS) cells, a high-virus-producer cell line derived from P3HR-1 cells by gradual adaptation to growth in low-serum medium (1.5% newborn calf serum) (12). Under these conditions, approximately 20 to 50% of the cells are spontaneously activated to produce virus and large numbers of linear EBV DNA genomes (unpublished data). Exponentially growing P3HR-1 (LS) cells were treated for 14 days with various concentrations of AZT in RPMI 1640 medium containing 1.5% serum. The cells were harvested, and EBV genome copy numbers were determined by complementary RNA-DNA hybridization with an EBV-specific cRNA probe.

Figure 1 shows the dose-dependent inhibition of EBV genome replication by AZT. EBV genome copy numbers decreased with increasing drug concentrations. The virus 50% effective dose (ED_{50}) and ED_{90} were determined from the semilogarithmic plot of drug concentrations against viral genome copies per cell, assuming the residual genome level (30 copies per cell) achieved by an effective drug concentration (100 μM) as zero and the viral genome level in the drug-free control as 100. We have shown previously that the residual EBV copy number of ~30 per cell is due to episomal forms which are insensitive to antiviral drugs (16). The ED_{50} and ED_{90} thus obtained were 3 and 30 μM, respectively. For comparison, in patients with acquired immunodeficiency syndrome (AIDS) the plasma concentration of AZT reaches levels of 6 to 10 μM 1 h after an intravenous dose of 5 mg/kg.

We considered that the EBV-inhibitory effect of AZT could be the consequence of selective killing of the productive cells in P3HR-1 (LS) populations, and therefore, we monitored cell growth and viability during drug treatment. Cell growth was not significantly affected at drug concentrations as high as 50 μM. At 100 μM AZT, approximately 30% growth inhibition was observed; however, the viability of the cells remained the same as that of the control. These results indicate that AZT at concentrations as high as 100 μM is cytostatic but not cytotoxic.

Since all herpesviruses induce the formation of new viral DNA polymerases, we thought that drugs inhibitory to EBV might also inhibit HSV, VZV, and HCMV replication (13). We therefore tested the effect of AZT on the replication of these viruses by using plaque-reduction assays (3); 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) was used as a positive drug control. As expected, DHPG exhibited potent anti-HCMV, -HSV-1 and -HSV-2 activities, with ED_{50}s ranging from 0.05 to 1.5 μM (Table 1). Surprisingly, AZT had no appreciable effect on HCMV, VZV, or HSV-1 and HSV-2.

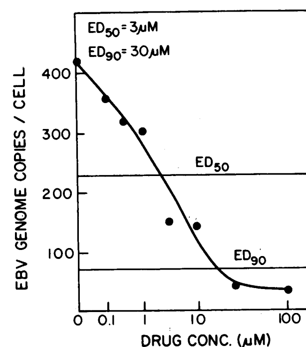

FIG. 1. Dose-dependent inhibition of EBV genome replication by AZT. Exponentially growing P3HR-1 (LS) cells were seeded at a density of 10^6/ml and incubated in various concentrations of drugs for 14 days. EBV genome copy numbers per cell represent the average of two determinations at each drug concentration.

* Corresponding author.

TABLE 1. Effects of AZT and DHPG on herpes-group viruses

Virus (strain)	ED_{50} (μM)	
	DHPG	AZT
HSV-1 (KOS)	0.05	>100
HSV-2 (333)	0.2	>100
HCMV (AD169)	1.5	>100
VZV (Oka)	ND[a]	>250

[a] ND, Not determined.

TABLE 2. Combined effects of AZT and ACV on EBV replication

Concn of ACV (μM)	EBV genome copy no. at AZT concn (μM)		
	0	2.5	10
0	465	382	116
1	216	133	80
5	82	62	62

To assess whether the inhibitory effect of AZT on EBV replication was reversible, we treated P3HR-1 (LS) cells with 50 μM AZT for 14 days to reduce the viral genome copy numbers down to the residual levels (30 copies per cell) and then released the cells into drug-free medium. Figure 2 shows that the inhibitory effect was slowly reversed upon removal of the drug. Replication of approximately 50% of the viral genome numbers was recovered by 14 days after drug removal. It required 21 days for the level of viral genomes to be restored to the control level; this result is in contrast to that obtained with acyclovir (ACV), the effect of which was completely abolished 11 days after removal of the drug (16). Slow kinetics of recovery have been demonstrated with other anti-EBV agents such as DHPG, *E*-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine, and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (14).

Because patients with AIDS have toxic reactions to AZT, combinations of drugs that are potentially synergistic and would permit reduction of AZT dosage are being sought. Synergistic inhibition of HIV replication in vitro by suramin and ACV (22), by phosphonoformate and alpha-A-interferon (11), and by ACV and AZT (19) has been reported, and a combination AZT-ACV therapy in AIDS is being evaluated clinically (S. Broder, personal communication). We looked for evidence of a potentiating effect between AZT and ACV on the replication of EBV but found only an additive effect when the two drugs were used in combination in suboptimal concentration (Table 2). The ED_{50} and ED_{90} of ACV alone for EBV replication are 0.3 and 9 μM, respectively (16).

The mechanism of selective inhibition by AZT on EBV replication is not understood. Initial preferential phosphorylation in virus-infected cells is a prerequisite for selective activity of several nucleoside analogs (for a review, see reference 13). The monophosphorylated compounds are converted by host cellular kinases to triphosphates, which, in turn, become preferential substrates for virus-specific DNA polymerase (5). In HIV-infected cells, initial phosphorylation of AZT is carried out by cellular thymidine kinase rather than viral enzyme, and the levels of AZT-monophosphate measured in uninfected and infected cells are similar (7). Thus, EBV-encoded thymidine kinase is unlikely to be required for the selective inhibition of EBV replication by AZT.

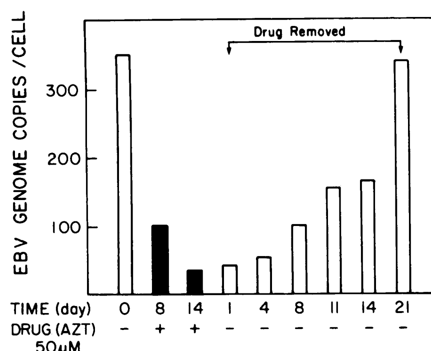

FIG. 2. Kinetics of inhibition and reversibility of EBV DNA replication in P3HR-1 (LS) cells treated with AZT.

Of special interest are the findings that AZT has virtually no effect on HCMV, VZV, and HSV-1 and HSV-2, despite the fact that all herpesviruses encode novel DNA polymerases that are quite distinct from cellular DNA polymerases and which share unique biochemical properties such as stimulation by high-salt concentrations in vitro. Moreover, there is extensive conservation of sequence of herpesvirus DNA polymerase genes (2). Indeed, in the predicted sequence of EBV and HSV-1 polymerases, there is 45% homology in the C-terminal two-thirds of the polypeptides (8). All of the known antiherpetic drugs generally affect several of the herpes-group viruses, although HCMV is insensitive to ACV (18) and HSV-2 is insensitive to BVDU (4).

Since AZT-triphosphate is a potent inhibitor of HIV reverse transcriptase (7), the question arises whether EBV DNA polymerase shares with the reverse transcriptase some common binding sites for the phosphorylated drug. If so, these sites are not available in α-polymerase, since AZT-triphosphate has low affinity for this cellular enzyme (7). In addition, treatment of EBV-infected cells with AZT may cause a reduction in the intracellular level of dTTP, which is a competing substrate for EBV DNA polymerase. This effect would facilitate the binding of AZT-triphosphate to viral polymerase. The structural characteristics of AZT with its 3′-azido group would also cause termination of DNA elongation if the nucleoside moiety were incorporated into viral DNA. In any case, the unique susceptibility of EBV to AZT may provide a lead toward ascertaining active sites on HIV reverse transcriptase for binding of the drug.

EBV has been suggested as one of the cofactors which determine whether AIDS will result from infection by HIV (9, 23). Since opportunistic viral infections, especially EBV and HCMV, are prevalent in AIDS (1, 24) treatment of symptomatic HIV infection with AZT may have an additional benefit in reducing possible facilitative effects or complications resulting from reactivated or primary EBV infection; however, this possibility has not yet been studied directly. Although the combination of ACV and AZT is not synergistic against EBV, neither is it antagonistic (25). Whether the EBV-associated B-lymphocytic lymphomas that are relatively common in AIDS would be affected is a matter of speculation (10).

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