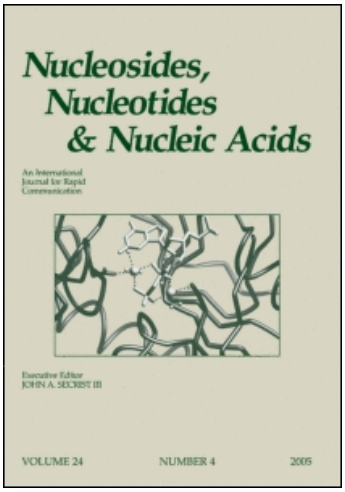

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COMBINATION OF AZIDOTHYMIDINE (AZT) AND (E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE (BVDU) INHIBITS THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) AND TYPE 2 (HSV-2) AND VARICELLA ZOSTER VIRUS (VZV) STRAINS THAT ARE DEFICIENT IN THE EXPRESSION OF THE VIRAL THYMIDINE KINASE (TK)

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Abstract. Combinations of high concentrations of AZT with BVDU, acyclovir (ACV) or ganciclovir (GCV) show antagonism against TK^+ HSV-1, but not TK^+ VZV strains, in cell cultures. When BVDU and AZT were used in combination against TK^- HSV-1, TK^- HSV-2 and TK^- VZV strains, a pronounced inhibition of viral replication was observed. This potentiating effect was not seen if AZT was combined with ACV or GCV.

INTRODUCTION

Although BVDU is a potent inhibitor of VZV and HSV-1, it has been shown to inhibit the replication of HSV-2 to a much lesser degree.[1] BVDU is phosphorylated to the mono- and diphosphate by the viral encoded TK and it is postulated to be triphosphorylated by cellular kinases.[2] Therefore, it is not active against TK^- HSV-1 and TK^- VZV strains that are deficient in the expression of the viral TK. Once activated to its triphosphate form, BVDU interacts with the viral DNA polymerase thus leading to inhibition of viral DNA synthesis. AZT is the reference compound for the treatment of patients suffering from AIDS, and as for other dideoxynucleosides, the triphosphate of AZT inhibits HIV reverse transcriptase.[3] The combination of AZT with BVDU and related compounds was evaluated against wild-type and TK^- strains of HSV-1, HSV-2 and VZV.

MATERIALS AND METHODS

Cells. Human embryonic lung (HEL) fibroblasts were used in the different experiments.

Virus. The wild-type VZV strains Oka and YS, the TK^- VZV reference strains YS-R and 07-1, two TK^- VZV clinical isolates (isolate III and isolate IV), the wild-type HSV-1 strain KOS and HSV-2 strain G, the TK^- HSV-1 reference strain B2006 and two HSV-2 clinical strains HS-47 (wild-type) and HS-44 (TK^-) were used.

Compounds. The following compounds were examined: BVDU [(*E*)-5-(2-bromovinyl)-1-(D-2-deoxyribofuranos-1-yl)uracil], Dr. R. Busson and Prof. P. Herdewijn, Rega Institute for Medical Research, Leuven, Belgium; ACV [acyclovir, 9-(2-hydroxyethoxymethylguanine], Wellcome Research Laboratories, Research Triangle Park, NC; DHPG [ganciclovir, GCV, 9-(1,3-dihydroxy-2-propoxymethyl)guanine], Syntex Research, Palo Alto, CA; BVaraU [1-ß-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil], Yamasa Shoyu Co., Choshi, Japan; CVDC [(*E*)-5-(2-chlorovinyl)-2'-deoxycytidine] and CVDU [(*E*)-5-(2-chlorovinyl)-2'-deoxyuridine], Dr. A. Kumar, Department of Chemistry, University of Birmingham, Birmingham, United Kingdom; EDU (5-ethyl-2'-deoxyuridine) and CEDU [5-(2-chloroethyl)-2'-deoxyuridine], Dr. B. Rosenwirth, Sandoz Forschungsinstitut, Wien, Austria; AZT (azidothymidine, 3'-azido-3'-deoxythymidine), ddI (dideoxyinosine), ddC (dideoxycytidine), didehydrodideoxythymidine (d4T) and thymidine (Thd), Sigma Chemical Co., St. Louis, MO.

Antiviral activity assays. Cells, grown in 96-cell microtiter plates, were inoculated with virus at an input of 20 PFU (VZV) or 100 $CCID_{50}$ (HSV). The assays were performed as previously described.[4,5] The 50% inhibitory concentration (IC_{50}) was defined as the concentration required to reduce virus plaque formation or cytopathicity by 50%.

Virus yield. HEL cells grown in chamber slides (2 wells) were inoculated with KOS strain (TK^+) or B2006 strain (TK^+) at a moi of 0.1. Two days after virus infection, the medium was harvested and the virus titers were determined by a plaque assay in HEL confluent monolayers.

RESULTS AND DISCUSSION

AZT was found to diminish the activity of BVDU (Table 1), ACV and GCV (data not shown) against HSV-1 (KOS strain). A similar decrease in the anti-HSV-1 activity of these drugs was observed when they were combined with deoxythymidine (dThd). However, AZT did not significantly affect the activity of BVDU (Table 2), ACV and GCV against TK^+ VZV strains. When the combination of BVDU with AZT was used against the replication of TK^- HSV-1, TK^- HSV-2 and TK^- VZV strains, there was a marked inhibition of viral replication, while none of the drugs alone afforded any activity against these virus strains. In the presence of AZT 100 μg/ml, the activity of BVDU was increased by 50- to 100-fold, reaching IC_{50} values of 0.5-1 μg/ml (Tables 1 and 2). Similar results were obtained with different TK^- HSV-1, TK^- HSV-2 and TK^- VZV strains. In contrast, AZT did not significantly affect the activity of ACV and GCV against TK^- strains of HSV and VZV. Surprisingly, no potentiated antiviral effect was seen if AZT was combined with molecules closely related to BVDU (such as BVaraU, CEDU, EDU, CVDC and CVDU). Similarly, when AZT was replaced by other dideoxynucleoside analogues (i.e. ddI, ddC or d4T), no synergy with BVDU was observed against any of the TK^- virus strains tested. That the combination of AZT with BVDU resulted in an antagonistic

TABLE 1

Activity of BVDU when combined with AZT on the *in vitro* replication of various TK^+ HSV and TK^- HSV strains

Treatment	IC_{50} (μg/ml)[a]				
	KOS (TK^+ HSV-1)	B2006 (TK^- HSV-1)	G (TK^+ HSV-2)	HS-47 (TK^+ HSV-2)	HS-44 (TK^- HSV-2)
BVDU	0.008	> 20	> 50	> 50	> 50
BVDU + AZT 100 μg/ml	0.2	0.4	> 50	> 50	1
BVDU + AZT 50 μg/ml	0.1	0.28	> 50	> 50	0.8
BVDU + AZT 2.5 μg/ml	0.0125		> 50	> 50	> 50

[a]Concentration required to reduce virus cytopathicity by 50% in HEL cells.

TABLE 2

Activity of BVDU when combined with AZT on the *in vitro* replication of various TK^+ VZV and TK^- VZV strains

Treatment	IC_{50} (μg/ml)[a]					
	Oka (TK^+)	YS (TK^+)	07-1 (TK^-)	YS-R (TK^-)	Isolate III (TK^-)	Isolate IV (TK^-)
BVDU	0.0008	0.0010	15	> 20	> 20	8
BVDU + AZT 100 μg/ml	0.0012	0.0050	0.44	0.94	0.39	0.31
BVDU + AZT 50 μg/ml	0.0010	0.0031	0.46	1	0.42	0.17
BVDU + AZT 2.5 μg/ml	0.0010	0.0021	10.5	> 20	> 20	1.25

[a]Concentration required to reduce virus-plaque formation by 50% in HEL cells.

TABLE 3

Virus yield production in TK^+ HSV-1(KOS)- or TK^- HSV-1(B2006)-infected HEL cells measured on day 2 p.i. after treatment with BVDU alone or in combination with AZT or dThd

Strain	Treatment	Log PFU/ml		
		-	AZT 100 μg/ml	dThd 100 μg/ml
TK^+ HSV-1(KOS)	-	4.1	4.1	5.1
	BVDU 0.04 μg/ml	0.2	2.5	3.6
	BVDU 0.01 μg/ml	2.1	5.0	5.0
TK^- HSV-1(B2006)	-	5.4	5.1	5.8
	BVDU 40 μg/ml	3.8	< 0.2	5.8
	BVDU 10 μg/ml	4.1	1.1	5.7

activity against the TK^+ HSV-1 KOS strain and synergistic action against the TK^- HSV-1 B2006 strain was confirmed by a virus yield assay (Table 3).

Since AZT is a poor substrate for phosphorylation by the HSV TK, but efficiently phosphorylated to AZT monophosphate by the cellular TK, the antagonistic effect observed between AZT and either ACV, BVDU or GCV against TK^+ HSV-1 may arise from direct competition between the monophosphates for further phosphorylation. The fact that the antiviral effects of ACV, BVDU and GCV are also reversed following the addition of dThd, the natural substrate for TK, supports this hypothesis. The mechanism of the synergy between AZT and BVDU against TK^- HSV-1, TK^- HSV-2 and TK^- VZV strains is now under investigation.

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