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Combination of Azidothymidine (AZT) with (*E*)-5-(2-Bromovinyl)-2'-Deoxyuridine (BVDU) Leads to Inhibition of the *in vitro* Replication of Herpes Simplex Virus Type 1 (HSV-1), Type 2 (HSV-2) and Varicella-Zoster Virus (VZV) Strains that Are Deficient in Thymidine Kinase (TK) Activity

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Although BVDU is a potent inhibitor of VZV and HSV-1, it inhibits the replication of HSV-2 to a much lesser degree. BVDU is phosphorylated to the mono- and diphosphate forms by the viral encoded TK; therefore it is not active against TK^- HSV-1 and TK^- VZV strains. AZT, like other dideoxynucleosides, is targeted at the HIV reverse transcriptase, and does not show activity against herpesviruses in human embryonic lung (HEL) fibroblasts. We have shown that high concentrations of AZT antagonize the activity of BVDU against TK^+ HSV-1 by an order of magnitude similar to that observed when BVDU is combined with deoxythymidine (dThd). However, AZT does not reduce the potent activity of BVDU 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 proved active against these viruses. No potentiating effect was seen if AZT was combined with molecules closely related to BVDU, i.e. (*E*)-5-(2-bromovinyl)-1-arabinofuranosyluracil (BVaraU), (*E*)-5-(2-chlorovinyl)-2'-deoxyuridine (CVDU), (*E*)-5-(2-chlorovinyl)-2'-deoxycytidine (CVDC), (*E*)-5-(2-chloroethyl)-2'-deoxyuridine (CEDU) and 5-ethyl-2'-deoxyuridine (EDU) or inhibitors of thymidylate synthase (i.e. fluorodeoxyuridine or trifluorothymidine). Also, when AZT was replaced by other dideoxynucleoside analogues [such as dideoxyinosine (ddI), dideoxycytidine (ddC) or didehydrodideoxythymidine (d4T)], no synergy with BVDU was observed against any of the TK^- virus strains tested. The mechanism of the potentiating effect of AZT on the activity of BVDU against TK^- herpesvirus strains remains to be elucidated.

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Analysis of mutations in the thymidine kinase gene of varicella-zoster virus associated with resistance to 5-iodo-2'-deoxyuridine (IDU) and 5-bromo-2'-deoxyuridine (BrDU).

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We analyzed the TK peptide sequence of VZV which has shown resistance only to 5-iodo-2'-deoxyuridine (IDU) and 5-bromo-2'-deoxyuridine (BrDU) whereas is susceptible to the other nucleoside analogs such as ACV, BV-araU, and BVDU. This virus strain, termed Ito strain, was isolated from a patient with chicken pox, and proved to be lowered in TK affinity to IDU and BrDU as substrate (Shigeta et al 1986 Antimicrob. Agents Chemother. 29, 1053-1058). To analyze the TK gene mutations, we carried out polymerase chain reaction (PCR) amplification and the whole TK gene was analyzed for the nucleotide sequence. Through sequencing of the TK gene, we found 3 amino acids were exchanged (41 N to S, 266 C to I, 288 S to L). These mutations were not located in neither nucleoside binding site nor ATP biding site. This result may suggest that the resistance for IDU and BrDU in this particular strain is due to the change in conformation of TK rather than the replacement of amino acids in the biding sites.