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Synergistic Antiviral Effects of Antiherpes Compounds and Human Leukocyte Interferon on Varicella-Zoster Virus In Vitro

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The four antiherpes compounds acyclovir, adenine arabinoside, bromovinyldeoxyuridine, and phosphonoformic acid showed an additive to synergistic effect with human leukocyte interferon in inhibiting focus formation by three different strains of varicella-zoster virus in human embryonic fibroblasts.

Varicella-zoster virus (VZV) is the causative agent of chickenpox (varicella) and shingles (herpes zoster). These are normally self-resolving diseases, but they are often severe and occasionally fatal in immunocompromised patients. We have recently examined several newly developed antiherpes compounds for their inhibitory effects on focus formation by VZV in vitro (9). The compounds varied widely in their potency and selectivity as anti-VZV agents, one of the most potent and selective being bromovinyldeoxyuridine (BVDU). Other well-known antiherpes drugs such as acyclovir (ACV), adenine arabinoside (ara-A), and phosphonoformic acid (PFA) proved less potent and less selective in their anti-VZV activity than BVDU did (2, 9).

Combinations of antiviral drugs are of interest because of their potential for increasing efficacy or reducing toxicity or both. Combinations of a number of agents, including ara-A and ACV (1, 6, 7, 11), have been evaluated against herpes simplex virus type 1 and type 2 in vitro. Combinations of antiviral agents at nontoxic concentrations usually result in synergistic, additive, subadditive, or indifferent effects, but rarely in antagonistic effects. Combinations including ACV generally result in an additive effect against VZV (3). ACV in combination with human interferon displays additive to synergistic effects against herpes simplex virus type 1 and type 2 (8, 10). In the present study, we examined the activities of combinations of human leukocyte interferon (HuIFN-α) with ACV, ara-A, PFA, or BVDU against VZV.

The sources of the antiherpes compounds were as follows: BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine], R. Busson and H. Vanderhaeghe, Rega Institute for Medical Research, Louvain, Belgium; ACV [9-(2-hydroxyethoxymethyl)guanine], Wellcome Research Laboratories, Research Triangle Park, N.C.; ara-A-[9-β-D-arabinofuranosyladenine], Parke Davis & Co., Ann Arbor, Mich.; PFA, Astra Läkemedel AB, Södertälje, Sweden. HuIFN-α was kindly provided by M. Krim, Sloan-Kettering Institute for Cancer Research, New York, N.Y. (degree of purity, $\geq 10^6$ U/mg of protein).

Three VZV strains were used. The CaQu strain was kindly provided by N. J. Schmidt, Viral and Rickettsial Disease Laboratories, Department of Health, Berkeley, Calif. The Hirai and Tokumaru strains were isolated in our laboratory from patients with herpes zoster and were passaged several times in human embryo fibroblast (HEF) cultures. All VZV strains were prepared as cell-free virus and were stored at −80°C until used. Antiviral assays were carried out in confluent HEF monolayers in Linbro multiwell plates with 24 flat-bottom wells (16-mm diameter). HEF cell cultures were propagated in Eagle minimal essential medium supplemented with 10% heat-inactivated fetal calf serum, 100 μg of streptomycin per ml, and 100 U of penicillin G per ml. After 3 days of cultivation, the HEF cultures were exposed for 24 h to various concentrations of HuIFN-α (0.25, 0.5, 1, 2, 4, 8, and 16 IU/ml) in maintenance medium (Eagle minimal essential medium containing 2% fetal calf serum and antibiotics at the concentrations mentioned above). After the HuIFN-α treatment, the HEF cultures were washed thoroughly with maintenance medium, infected with ca. 50 PFU of VZV (multiplicity of infection, 5×10^{-4}), and immediately thereafter exposed to various concentrations of the compounds (BVDU, 0.0025, 0.005, 0.01, 0.02, and 0.04 μg/ml; ACV or ara-A, 0.25, 0.5, 1, 2, and 4 μg/ml; PFA, 5, 10, 20, 40, and 80 μg/ml) in maintenance medium. Foci were counted microscopically at 3 days after virus infection without fixation and staining of cells. Dose-response curves for all antiviral compounds were set up in combination with various concentrations of HuIFN-α, and the concentration of compound (or HuIFN-α) required to reduce the number of foci to 30% of that in the control cell cultures (infected with VZV but not exposed to either antiviral compound or HuIFN-α) was chosen as the endpoint of antiviral activity. When focus reduction was not exactly 30% of the control, the endpoint concentration was obtained by plotting the number of foci as a function of the compound concentration (Fig. 1). Data were plotted and analyzed by the isobologram method (5). The fractional inhibitory concentration (FIC) for each pair, i.e., compound x plus HuIFN-α, was calculated as follows: FIC_x = (concentration of x in the combination at the endpoint)/(concentration of x alone required to achieve that endpoint) and FIC_{IF} = (concentration of HuIFN-α in the combination at the endpoint)/(concentration of HuIFN-α alone required to achieve that endpoint). Combinations resulting in additive antiviral ($FIC_x + FIC_{IF} = 1$) are represented by straight lines (unity lines) on the isobolograms (dotted in Fig. 2). When the combination resulted in synergy, a stronger antiviral effect than the sum of the individual effects ($FIC_x + FIC_{IF} < 1$), the representational line shifts below the unity line. When the combination resulted in antagonistic activity ($FIC_x + FIC_{IF} > 1$), the line shifts above the unity line.

The dose-response curves for the different antiherpes compounds are shown in Fig. 1. All compounds effected a significantly greater reduction in VZV focus formation after combination with HuIFN-α. Experiments similar to that shown for the CaQu strain (Fig. 1) were carried out with the Hirai and Tokumaru strains (data not shown).

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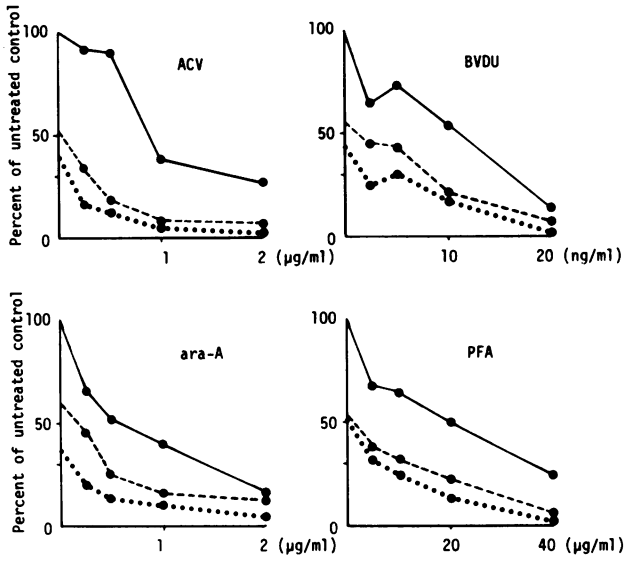

FIG. 1. Dose-response curves for ACV, BVDU, ara-A, and PFA, each in combination with HuIFN-α, against the CaQu strain of VZV. The concentrations of ACV, BVDU, ara-A, and PFA are indicated on the abscissa. Symbols: solid line, compound alone; broken line, compound combined with 1 U of HuIFN-α per ml; dotted line, compound combined with 2 U of HuIFN-α per ml.

Only two representational lines (Fig. 2), namely those for the combinations BVDU–HuIFN-α and ara-A–HuIFN-α against the Hirai strain of VZV, merely followed the unity line (i.e., indicated additive antiviral activity). Two other lines for the BVDU–HuIFN-α and PFA–HuIFN-α combinations showed additive to subsynergistic effects against the Tokumaru strain. For all other VZV strains and drug combinations, in particular the ACV–HuIFN-α combination, antiviral activity was clearly synergistic.

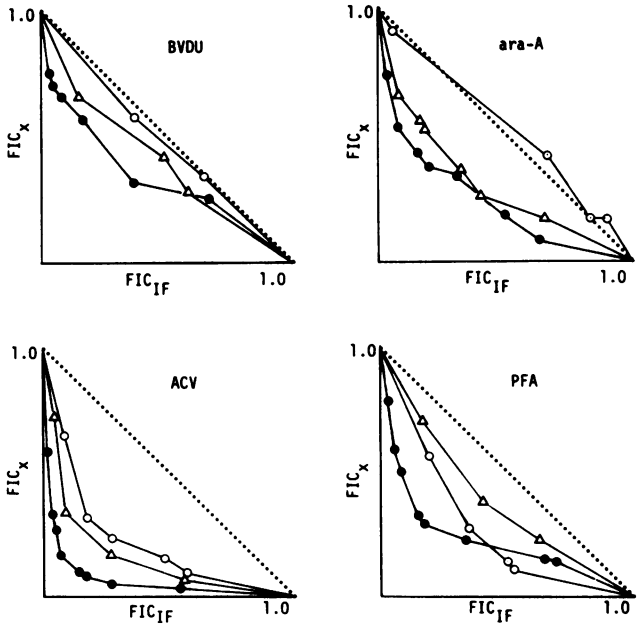

FIG. 2. Isobologram representation of drug combinations. Symbols: ●, CaQu strain; ○, Hirai strain; △, Tokumaru strain; dotted line, unity line.

TABLE 1. Minimal FICs (FIC_x + FIC_{IF}) for four antiviral compounds when combined with HuIFN-α

Compound	Minimal FIC for strain:			Mean minimal FIC
	CaQu	Hirai	Tokumaru	
BVDU	0.66	0.94	0.79	0.80
ACV	0.22	0.48	0.42	0.37
Ara-A	0.55	0.97	0.63	0.72
PFA	0.47	0.60	0.75	0.61

To compare the antiviral effects of the test compounds and HuIFN-α further, we determined the minimal FICs (FIC_x + FIC_{IF}) for each VZV strain (Table 1). From these results it is clear that all combinations resulted in additive to synergistic activity against VZV, with the degree of synergism decreasing in the order ACV–HuIFN-α > PFA–HuIFN-α > ara-A–HuIFN-α BVDU–HuIFN-α. Thus, of all four antiherpes compounds tested, ACV gained the most in antiviral potency from combination with HuIFN-α. A similar degree of synergism between ACV and HuIFN-α against focus formation of VZV was reported by Levin and Leary (8). Also, additive to synergistic anti-VZV effects have been reported previously for combinations of BVDU with ACV and ara-A (Y. Bryson, D. Hebblewaite, and E. De Clercq, Internat. Congr. Chemother. 12th, Florence, Italy, abstract 131, 1981).

The mechanism of the synergistic action between interferon and the antiherpes drugs, as well as the performance of these drug combinations in vivo, remains to be determined. The selective antiherpes activity of compounds such as ACV and BVDU depends primarily on a specific phosphorylation by the virus-encoded thymidine kinase (2, 4, 9). Apparently, pretreatment with HuIFN-α does not alter this specific viral function. Otherwise, one should expect a diminution in the antiviral effects of ACV and BVDU. Furthermore, combination of HuIFN-α (64 U) with the antiviral drugs ACV, BVDU, ara-A, and PFA (at 100 μg/ml) did not cause an increase in the cytotoxicity of the latter when examined by the trypan blue exclusion test (data not shown). This points to the clinical potential of such combinations in the therapy, prophylaxis, or both of herpesvirus infections.

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