Inhibitory Effects of Antiviral Thymidine Analogs Against Varicella-Zoster Virus

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Thymidine analogs highly active against herpes simplex virus were compared in their inhibitory action against seven strains of varicella-zoster virus by a plaque reduction assay. E-5-Bromovinyl-arabinosyluracil (BV-ara-U) was most active, followed by E-5-Chlorovinyl-arabinosyluracil, E-5-Bromovinyl-2'-deoxyuridine (BV-dUrd), 2'-fluoro-5-methyl-arabinosyluracil, 2'-fluoro-5-iodo-arabinosylcytosine, arabinosylthymine, 5-vinyl-arabinosyluracil, acycloguanosine, and 5-iodo-2'-deoxyuridine, in order of decreasing activity. BV-ara-U was more than 10 times as active as BV-dUrd and almost completely inhibited plaque development of five strains of varicella-zoster virus at a concentration as low as 1 ng/ml.

Infection with varicella-zoster virus (VZV), a member of the human herpesvirus group, in normal individuals is generally a self-resolving, uncomplicated disease. However, complications resulting from VZV infection can be severe to life threatening in immunocompromised patients (5, 14, 17). It is necessary to develop nontoxic and effective antiviral agents for treatment of severe VZV infection. The object of this paper is to show that 1-β-D-arabinofuranosyl-5-bromovinyluracil (BV-ara-U) and 1-β-D-arabinofuranosyl-5-chlorovinyluracil (CV-ara-U) were extraordinarily active against seven strains of VZV.

Human embryonic lung fibroblast (HEL-F) cells were used in this study. Methods for cultivation of the cells were described previously (10). VZV strains YS and Asahikawa were kindly supplied by T. Sakuma, Asahikawa Medical College, Asahikawa, Japan; strains Oka and Kawaguchi were supplied by M. Takahashi, Research Institute for Microbial Disease, Osaka University, Osaka, Japan; and strains Kanno, Hirai, and Ohtomo were supplied by S. Shigeta, Fukushima Medical College, Fukushima, Japan. These strains of VZV were freshly isolated from patients and passed 6 to 20 times in HEL-F cells, except for strain Ohtomo, which was passed more than 100 times in HEL-F cells.

A plaque reduction method was employed for the determination of activity against VZV. Confluent monolayers (4 to 6 days old) of HEL-F cells grown in a multiwell plate (Linbro FB-12-TC) were infected with about 50 PFU of VZV in 0.5 ml of maintenance medium per well. Either cell-free virus preparation (strains Oka and Kawaguchi) or cell-associated virus preparation (other strains) was employed. At 2 h after infection, 0.5 ml of maintenance medium containing an appropriate amount of the test compound was added to the infected cultures in duplicate for each dilution. The infected cells were incubated at 37°C for 6 to 8 days, and then the number of plaques in each well was counted microscopically without staining.

The following compounds were tested for their antiviral activity against VZV: 1-β-D-arabinofuranosyl-5-ethyluracil (ethyl-ara-U) (10), 1-β-D-arabinofuranosyl-5-vinyluracil (vinyl-ara-U) (8), BV-ara-U, CV-ara-U (9), E-5-(2-bromovinyl)-2'-deoxyuridine (BV-dUrd) (4), 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FI-ara-C), 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FM-ara-U) (18), and acyclovir (ACV) (16). These compounds had been shown to exhibit high activity against herpes simplex virus (HSV). BV-dUrd, FI-ara-C, FM-ara-U, and ACV were kindly supplied by E. De Clercq, Katholieke Universiteit Leuven, Leuven, Belgium; by J. J. Fox, Sloan-Kettering Institute, New York; and by W. G. Wilson, Nippon Welcome Co. Ltd., Shinoo, Osaka; respectively. 5-Iodo-2'-deoxyuridine (I-dUrd) and ara-T, in decreasing order. It is notable that products of Yamasa Shoyu Co., Ltd.

For microscopic observation, clear and large plaques of all strains of VZV were developed in drug-untreated control cultures within 4 to 6 days after infection. At that time, however, a marked decrease in plaque size and incomplete plaque development were observed in cultures treated with the drugs at approximately minimum inhibitory concentrations or more. To be sure of the number of plaques in the drug-treated cultures, plaque development was allowed to continue for an additional 2 to 4 days. Even after
such treatment, the plaque sizes in the drug-treated cultures were often much smaller than those in control cultures 4 to 6 days after infection. A marked reduction in number of plaques was always accompanied by a reduction in plaque size in the cultures treated with any of the drugs tested. In accord with our observation, previous studies have shown that treatment with 9-β-d-arabinofuranosyladenine or ACV results in slower development of plaques of VZV or reduction in its plaque size at concentrations below the minimum inhibitory concentration (1, 6). In the present study, we noted a reduction in number of plaques only in drug-treated cultures.

As shown in Table 1, BV-ara-U was most active against all strains of VZV, followed by CV-ara-U, FM-ara-U, BV-dUrd, FI-ara-C, and ara-T, in decreasing order. It is notable that although the degree of inhibition of a certain strain by each drug was not always the same as that of other strains, the order of the analogs in the inhibitory action was almost identical irrespective of the strain employed. BV-ara-U and CV-ara-U inhibited almost completely the plaque development of four or five out of seven strains tested at a concentration as low as 1 ng/ml, whereas 10 ng of BV-dUrd or FM-ara-U per ml was necessary to inhibit the strains to the same extent. Furthermore, BV-ara-U markedly inhibited the plaque development of the most

| Table 1. Inhibitory action of thymidine analogs to seven strains of VZV |
|---------------------------------------------------------------|
| **Compound** | **Concn (µg/ml)** | **Plaques (% of control of following strain:** |
|               |                  | YS | Asahikawa | Oka | Kawaguchi | Kanno | Hirai* | Ohtomo |
| Ethyl-ara-U    | 1,000            | 39 | 4        | 47  | 14        | 46   | 38    | 40    | 37    |
|                | 100              | 100| 62       | 100 | 100       | 71   | 53    | 79    | 48    |
|                | 10               | 100| 100      | 100 | 100       | 100  | 100   | 100   | 49    |
| ACV            | 10               | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     |
|                | 0.1              | 50 | 72       | 94  | 43        | 25   | 18    | 8     | 3     |
| I-dUrd         | 10               | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     |
|                | 0.1              | 43 | 33       | 45  | 0         | 75   | 38    | 69    | 3     |
| Vinyl-ara-U    | 1                | 56 | 31       | 16  | 9         | 3    | 0     | 0     | 0     |
|                | 0.1              | 97 | 86       | 66  | 82        | 42   | 8     | 29    | 32    | 0     |
| Ara-T          | 1                | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     |
|                | 0.1              | 100| 100      | 84  | 69        | 1    | 25    | 12    | 0     | 0     |
|                | 0.01             | 100| 100      | 100 | 100       | 66   | 76    | nd*   | 6     | 83    |
| FI-ara-C       | 0.1              | 14 | 19       | 0   | 18        | 2    | 0     | 0     | 1     | 0     |
|                | 0.01             | 90 | 74       | 93  | 89        | 85   | 33    | 20    | 48    | 5     | 24    |
|                | 0.001            | 100| 100      | 100 | 100       | 100  | 100   | 100   | 90    | 100   |
| FM-ara-U       | 0.1              | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     | 0     |
|                | 0.01             | 22 | 73       | 95  | 50        | 14   | 33    | 0     | 12    | 0     | 1     |
|                | 0.001            | 92 | 86       | 100 | 97        | 100  | 100   | 96    | 100   | 48    | 95    |
| BV-dUrd        | 0.1              | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     | 0     |
|                | 0.01             | 52 | 74       | 85  | 50        | 5    | 30    | 0     | 12    | 0     | 0     |
|                | 0.001            | 92 | 100      | 100 | 92        | 100  | 100   | 96    | 100   | 18    | 77    |
| CV-ara-U       | 0.1              | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     | 0     | 0     |
|                | 0.001            | 47 | 37       | 53  | 22        | 2    | 0     | 3     | 8     | 0     | 0     |
|                | 0.0001           | 100| 91       | nd  | 93        | 85   | 77    | 96    | 100   | 32    | 58    |
| BV-ara-U       | 0.1              | 0  | 0        | 0   | 0         | 0    | 0     | 0     | 0     | 0     | 0     |
|                | 0.001            | 23 | 4        | 21  | 3         | 0    | 0     | 3     | 8     | 0     | 0     |
|                | 0.0001           | 100| 78       | nd  | 100       | 67   | 31    | 72    | 96    | 15    | 36    |

* nd, Not done.
susceptible strain, Ohtomo, at a concentration as low as 0.1 ng/ml. Vinyl-ara-U was a little less effective than ara-T, although the former was more inhibitory than ara-T to HSV type 1 (HSV-1) (8, 9). Compared with these compounds, ACV and I-dUrd exhibited relatively low activities. This finding was not unexpected because ACV is reported to be significantly less active against VZV than against HSV (1, 2). Ethyl-ara-U, whose activity against HSV-1 was almost the same as that of I-dUrd (10), exhibited extremely low activity against all strains of VZV tested.

To examine the reproducibility of the assay, we retested the activities of almost all of the thymidine analogs against VZV strains Asahikawa, Hirai, and Ohtomo (Table 1, experiment 2). There was no marked discrepancy in the results from the two separate experiments. Especially, the order of the activities of the thymidine analogs was identical.

Although some thymidine analogs, such as ara-T (12), ACV (1, 2), and FI-ara-C (7), inhibit multiplication of VZV, the activities of BV-ara-U and CV-ara-U against VZV were greater than those of the representative compounds. In addition, the anti-VZV activity of BV-ara-U was much higher than that of BV-dUrd. Anti-HSV activity of BV-dUrd was weakened by replacement of its 2'-deoxyribose with arabinose (conversion of BV-dUrd to BV-ara-U) when tested in primary rabbit kidney cells (E. De Clercq, personal communication) or in Vero cells (G. Stening et al., Antiviral Res., in press). In a human cell system, anti-HSV activity of BV-ara-U is not superior to that of BV-dUrd (9). Anti-HSV activity of certain 5-substituted 2'-deoxyuridines was not reduced by replacement of their deoxyribose moiety with arabinose, but in many other cases the activity was partially or completely reduced by the replacement (11, 15). Our finding may be the first report showing that an ara-U derivative is much more active than the corresponding deoxyuridine derivative having the same substituent at the C-5 position.

As reported previously (9, 11), cytotoxicity of BV-ara-U and CV-ara-U was extremely low. They did not inhibit cellular metabolism at a concentration of 100 μg/ml in confluent monolayers of HEL-F cells. Neither DNA synthesis in exponentially growing HEL-F cells nor growth of the cells after 4 days of incubation was significantly inhibited by them at a concentration as high as 300 μg/ml. Even after 6 days of incubation with BV-ara-U, the cell growth was little more than that after 4 days of incubation (data not shown). It should be noted that a 6-day incubation was also employed here in the plaque reduction assays for VZV. The antiviral index (the 50% inhibitory dose for HEL-F cells divided by the 50% plaque reduction dose for VZV) of BV-ara-U can be estimated to be about 10^6 or more. Such a high value means that the inhibitory action of BV-ara-U is highly selective for VZV. Treatment with low toxic thymidine analogs, such as BV-dUrd (3) and ACV (13), caused prompt recovery of patients with severe herpes zoster. Clinical application of BV-ara-U seems to be more promising than that of BV-dUrd and ACV because BV-ara-U exhibited extremely low cytotoxicity and was more active than such representative analogs against VZV.

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ERRATA

Plasmid-Mediated Sulfonamide Resistance in *Haemophilus ducreyi*

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Volume 21, no. 1, p. 162, Fig. 3 legend, line 3: “respectively digested with *HincII* and *PstI*” should read “respectively digested with *HincII*.”

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Volume 21, no. 2, p. 358, column 2, line 26: should read “ara-T, control compounds, were commercial products of Yamasa Shoyu Co., Ltd.”