Selective Inhibition of Herpesvirus Deoxyribonucleic Acid Synthesis by Acycloguanosine, 2'-Fluoro-5-Iodo-Aracytosine, and (E)-5-(2-Bromovinyl)-2'-Deoxyuridine

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The selectivity of inhibition of herpesvirus deoxyribonucleic acid synthesis by acycloguanosine, 2-fluoro-5-iodo-aracytosine, and (E)-5-(2-bromovinyl)-2'-deoxyuridine was determined by isopycnic banding of 32P-labeled deoxyribonucleic acid from herpesvirus-infected and uninfected cells.

An important property of an antiviral compound is the selectivity of its action. Most compounds with a high activity against herpesviruses are nucleoside analogs (3, 8, 9, 11). Several of these analogs have not only inhibited virus multiplication in infected cells but also affected normal cell metabolism.

The selectivity of an antiviral compound whose primary site of action is viral deoxyribonucleic acid (DNA) synthesis has previously been determined by isopycnic separation of [3H]thymidine-labeled DNA in CsCl gradients (4). However, the use of radiolabeled thymidine as a precursor could be misleading if the tested compound interferes with phosphorylation of thymidine or competes with thymidine incorporation into DNA. To avoid these problems we have used [32P]orthophosphate to label newly synthesized DNA in the presence of inhibitors (A. Larsson and B. Öberg, J. Antiviral Res., in press). To measure both viral and cellular DNA synthesis, nonconfluent cell cultures and a low multiplicity of infection were used. The selective action of acycloguanosine (ACG), 2'-fluoro-5-iodo-aracytosine (FIAC), and (E)-5-(2-bromovinyl)-2'-deoxyuridine (BrVDU) on viral and cellular DNA synthesis in herpes simplex virus type 1 (HSV-1)-infected and uninfected Vero cells has been determined by isopycnic banding of DNA in CsCl gradients. These nucleosides have previously shown a high antiviral activity (1, 5, 10, 12, 13) and are also known to require the presence of herpesvirus-induced thymidine kinase for their antiviral activity (2, 5, 10).

ACG showed a high selectivity in the inhibition of viral DNA synthesis (Fig. 1A to C; Table 1). When viral DNA synthesis was inhibited by 90% at 0.3 μM, only 15% inhibition of cellular DNA synthesis was observed. No effect on DNA synthesis in uninfected cells could be detected at this concentration and 310 μM ACG was required for a 50% inhibition. The high selectivity found for ACG correlates well with earlier results showing that ACG is phosphorylated to a much higher extent to its mono-, di-, and triphosphate in HSV-1-infected cells than in uninfected cells and that ACG triphosphate is selectively accepted by HSV-1 DNA polymerase, leading to chain termination (5, 7).

FIAC was less selective than ACG in the inhibition of viral DNA synthesis in infected cells (Fig. 1D to F; Table 1). At 0.10 μM a 90% inhibition of viral DNA synthesis was obtained, whereas cellular DNA synthesis in infected cells was inhibited by 40%. However, DNA synthesis in uninfected cells was not inhibited at this concentration, and 64 μM FIAC was required to obtain a 50% inhibition, which confirms the results of Lopez et al. (10) showing a high selectivity by FIAC when effects on HSV-1 multiplication and cytotoxicity for uninfected Vero cells were compared.

BrVDU inhibited viral DNA synthesis by 90% at a concentration of 0.6 μM (Fig. 1G to I; Table 1). As in the case of FIAC, but not of ACG, the cellular DNA synthesis in infected cells was also affected, to 60%, at this concentration. In the uninfected cells, DNA synthesis was not affected by 0.6 μM BrVDU, and at 440 μM a 50% inhibition was observed. These results agree with those of De Clercq et al. (1), who have shown previously that BrVDU inhibits HSV-1 multiplication at low concentrations, while not affecting the growth of uninfected cells.

In a cell-free assay, BrVDU seems to be selectively phosphorylated by HSV-1 thymidine kinase (A. Larsson, unpublished data). BrVDU triphosphate was found to substitute for deoxythymidine 5'-triphosphate in the polymerase reaction directed by HSV-1 DNA polymerase and...
FIG. 1. Effects of ACG, FIAC, and BrVDU on DNA synthesis in HSV-1-infected Vero cells. Vero cells grown to about 80% confluence in 35-mm cluster dishes were infected with 1 to 2 plaque-forming units of HSV-1 (strain C42) per cell. The test compounds were added to the medium 1 h postinoculation, and [\(^{32}\)P]-orthophosphate (5 to 10 μCi/ml) was added 3 h postinoculation. The cells were harvested after 16 h of incubation, and cellular and viral DNA were separated in CsCl gradients. \([^{3}H]\)Thymidine-labeled DNA from infected and untreated cells was used as an internal density marker (not shown). The methods have been described previously (Larsson and Öberg, in press). The shaded areas show HSV-1 DNA. (A) Control; (B) 0.025 μM ACG; (C) 1.0 μM ACG; (D) control; (E) 0.025 μM FIAC; (F) 0.5 μM FIAC; (G) control; (H) 0.1 μM BrVDU; (I) 1.0 μM BrVDU.

### TABLE 1. Inhibition of HSV-1 and cellular DNA synthesis by ACG, FIAC, and BrVDU

| Compound | HSV-1-infected DNA | Cell DNA | Uninfected cell DNA |
|----------|-------------------|----------|---------------------|
|          | IC\(_{50}\) (μM) | IC\(_{90}\) (μM) | IC\(_{50}\) (μM) | IC\(_{90}\) (μM) | B/A | C/A |
| ACG      | 0.03              | 0.3      | >2                  | >2                  | 310  | >500 | >67 | 10.333 |
| FIAC     | 0.02              | 0.1      | 0.5                 | >2                  | 64   | >250 | 25  | 3.200  |
| BrVDU    | 0.1               | 0.6      | 0.2                 | >2                  | 440  | >500 | 2   | 4.400  |

* Seven different concentrations of each inhibitor were used to determine the 50 and 90% inhibitory concentrations (IC\(_{50}\), IC\(_{90}\)). DNA was banded in CsCl gradients, and the amount of radioactivity in each peak was determined as described for Fig. 1. The data are averages from three different experiments.

Cellular DNA polymerase α (B. Eriksson, B. Öberg, and K. K. Gauri, in Second International Symposium on Antiviral Chemotherapy, in press). These data, together with the limited selectivity of inhibition of HSV-1 DNA synthesis in infected cells (Table 1), indicate that the good antiviral effect of BrVDU is dependent mainly on a selective phosphorylation by HSV-1 thymidine kinase and not on a selective inhibition of HSV-1 DNA polymerase by BrVDU triphosphate.

A density shift was observed for HSV-1 DNA (not shown) after infected cells were treated with BrVDU, indicating a fairly extensive incorporation of BrVDU monophosphate into DNA, but no density shift was seen for cellular DNA. No density shift was observed for FIAC, where the presence of an iodine atom could increase the density. Incorporation of ACG should not lead to any detectable density shift, and none was observed.

The present results demonstrate that the selectivity, and to some extent the mode of action, of herpesvirus inhibitors can be determined by isopycnic separation of viral and cellular DNA labeled with \([^{32}\)P]orthophosphate. ACG, FIAC, and BrVDU all showed high selectivity when their effects on HSV-1 DNA synthesis were com-
pared with their effects on cellular DNA synthesis in uninfected cells. This is probably due to a preferential phosphorylation by HSV-1 thymidine kinase (2, 5, 10). ACG seemed to be the most selective nucleoside analog when the effects on HSV-1 and cellular DNA synthesis in infected cells were compared, indicating that its triphosphate is more selective for viral DNA polymerase than the triphosphates of FIAC and BrVDU.

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