Kinetics of the Interaction of Monophosphates of the Antiviral Nucleosides 2'-Fluoro-1-β-D-Arabinofuranosylpyrimidine and (E)-5-(2-Bromovinyl)-2'-Deoxyuridine with Thymidylate Kinases from Vero Cells and Herpes Simplex Virus Types 1 and 2

MING S. CHEN,* LEONARD A. AMICO, AND DAN J. SPEELMAN

Virology Department, Bristol-Myers Pharmaceutical Research and Development Division, Syracuse, New York 13221-4755

Received 29 May 1984/Accepted 8 August 1984

The affinities of the monophosphates of 2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil and its 5-methyl analog for cellular thymidylate kinase were two or more orders of magnitude greater than for the thymidine-thymidylate kinases from herpes simplex virus types 1 and 2. In contrast, the monophosphate of (E)-5-(2-bromovinyl)-2'-deoxyuridine was found to have a higher affinity for the viral enzymes than for the cellular enzyme.

Several nucleoside analogs developed during the past few years (7, 9, 17, 20) show great promise for the chemotherapy of herpes simplex virus, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus infections. These antiviral nucleoside analogs are preferential substrates for virally encoded thymidine-thymidylate kinase (1, 5). They exert their antiviral activity after being phosphorylated to their triphosphate derivatives, which inhibit viral DNA synthesis by competing with natural substrates (10, 11, 18). In some cases, the nucleoside analogs are also incorporated into viral DNA (10, 11, 13). The initial phosphorylation to their monophosphate derivatives is produced by viral thymidine-thymidylate kinase; however, the subsequent conversion to their diphosphate derivatives may depend upon either viral or host enzymes or both. The phosphorylation of acyclovir monophosphate to its diphosphate was shown to be a function of host cell guanylate kinase activity (15), whereas (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd) monophosphate was preferentially phosphorylated by herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella-zoster virus-encoded thymidine-thymidylate kinases (12). The HSV-2 thymidine-thymidylate kinase only poorly phosphorylates BVdUrd monophosphate to its diphosphate, distinguishing it from the enzymes of HSV-1 and varicella-zoster virus (12). The lower sensitivity of HSV-2 strains than of HSV-1 strains to BVdUrd inhibition was related to the lower levels of thymidylate kinase activity of the thymidine-thymidylate kinase found in HSV-2-infected cells. Although the rate of phosphorylation of BVdUrd monophosphate has been reported (12), the affinity of BVdUrd monophosphate for cellular and viral enzymes has not been described. Here we report the kinetic constants of the monophosphates of 2'-fluoro-1-β-D-arabinofuranosylpyrimidine nucleosides, BVdUrd, and definition (5-iodo-2'-deoxyuridine [IdUrd]) for thymidylate kinase from Vero cells and thymidine-thymidylate kinases from HSV-1 and HSV-2.

BVdUrd was supplied by I. S. Sim (G. D. Scarle, High Wycombe, Buckinghamshire, United Kingdom). 2'-Fluoro-1-β-D-arabinofuranosylpyrimidine nucleosides were synthesized at Bristol-Myers Pharmaceutical Research and Development Division, Syracuse, N.Y., by the procedure described by Watanabe et al. (20). The identities and purities of the compounds were analyzed by elemental analysis, nuclear magnetic resonance spectroscopy, and high-pressure liquid chromatography. The monophosphates of the various nucleoside analogs were prepared by the procedure of Yoshikawa et al. (21) and purified on a DEAE column (bicarbonate form). Their identities were confirmed by determining the ratio of phosphate to nucleoside and by nuclear magnetic resonance spectroscopy.

Thymidylate kinase from rapidly dividing Vero cells and thymidine-thymidylate kinases from LMTK- cells infected with HSV-1 (KOS strain) and HSV-2 (MS strain) were purified as described by Cheng and Ostrander (6) and Chen and Prusoff (3). Enzymes were changed to the ATP·Mg2+ form before being used in kinetic studies by passage through a Sephadex G-25-40 column (Sigma Chemical Co., St. Louis, Mo.) equilibrated with 1 mM ATP·Mg2+·10 mM Tris (pH 7.5)-10% glycerol and were eluted with the same buffer. The assay for enzyme activities and analysis of kinetic data were done as described previously (2, 4).

The kinetic constants of the monophosphates of the nucleoside analogs for thymidylate kinase from Vero cells and thymidine-thymidylate kinases from HSV-1 and HSV-2 are shown in Table 1. Thymidylate kinase from Vero cells showed a higher affinity for its normal substrate, thymidylate, than did the viral enzymes. The \( K_m \) for thymidylate for the HSV-2-encoded thymidine-thymidylate kinase was ca. 20-fold higher than for the enzyme from Vero cells. The monophosphate derivatives of 2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil and 2'-fluoro-1-β-D-arabinofuranosylthymine had a much higher affinity for the cellular enzyme than for the viral enzymes. In contrast, the monophosphate of 2'-fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAC) had a poor affinity for both the cellular and the viral enzymes, and no inhibition of the phosphorylation of thymidylate by the cellular enzyme could be detected in the presence of 900 \( \mu M \) FIAC monophosphate. FIAC monophosphate was a noncompetitive inhibitor against thymidylate in the HSV-1 and HSV-2 enzyme assays. The results with BVdUrd monophosphate as an inhibitor showed that the viral enzymes had a greater affinity for it than did the

* Corresponding author.
TABLE 1. Kinetic constants for thymidylate kinase purified from Vero cells and thymidine-thymylate kinases purified from HSV-1- and HSV-2-infected LMTK² cells

<table>
<thead>
<tr>
<th>Substrate or inhibitor</th>
<th>Kinetic constant (μM) for enzyme from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vero cells</td>
</tr>
<tr>
<td>dTMP</td>
<td>4.1</td>
</tr>
<tr>
<td>FIAU monophosphate</td>
<td>1.4</td>
</tr>
<tr>
<td>FMAU² monophosphate</td>
<td>0.3</td>
</tr>
<tr>
<td>FIAc monophosphate</td>
<td>&gt;900⁶</td>
</tr>
<tr>
<td>IdUrd monophosphate</td>
<td>15.5</td>
</tr>
<tr>
<td>BvdUrd monophosphate</td>
<td>400</td>
</tr>
</tbody>
</table>

⁶ Kₘ was used for dTMP. The affinity for fraudulent nucleotides was measured as Kₘ because the fraudulent nucleotides were not available in radiolabeled form.

² FIAU, 2'-Fluoro-5-iodo-1-β-D-arabinofuranosyluracil.

² FMAU, 2'-Fluoro-1-β-D-arabinofuranosylthymine.

² No inhibition could be detected at this concentration.

² Noncompetitive inhibition against thymidylate.

² NT, Not tested.

cellular enzyme, and the HSV-1 enzyme had the highest affinity for BvdUrd monophosphate of the three enzymes studied. BvdUrd has been reported to be about 100-fold less active against HSV-2 than against HSV-1 (8), and this is consistent with the levels of thymidylate kinase activity of the virus-encoded thymidine-thymylate kinase found in HSV-2 being 50-fold lower than those in HSV-1 (12). In addition, the HSV-2 thymidylate kinase also had a lower affinity for BvdUrd monophosphate than did the HSV-1 enzyme, and cellular thymidylate kinase had a poor affinity for BvdUrd monophosphate. The combination of these factors results in the relative resistance of HSV-2 to BvdUrd as compared with the resistance of HSV-1 to BvdUrd. The monophosphate of IdUrd showed a similar affinity for both the cellular and the viral enzymes. Thus, at the level of thymidylate kinase, IdUrd monophosphate has no selectivity for herpes simplex virus, and this may be reflected in the toxicity associated with this drug.

Although previous studies have indicated that FIAC has antiviral activity in cell cultures and animals (14, 16, 19), the presence of FIAC triphosphate has not been reported in either in vitro or in vivo systems (13). However, metabolites of FIAC (principally 2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil) have been shown to be incorporated into cellular and viral DNAs (13). All of the three enzymes tested in our study had a low affinity for FIAC monophosphate. These findings would suggest that either FIAC monophosphate is acted on by enzymes other than the thymidine-thymylate kinases studied here or that the antiviral activity of FIAC is dependent upon its deamination to 2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil at either the nucleoside or the monophosphate level. The observation that FIAC had no inhibitory activity against Marek’s disease virus in chicken embryo fibroblasts lacking 2'-deoxycytidine deaminase, whereas the virus was inhibited by FIAC in chicken kidney cells containing the enzyme, may support the latter hypothesis (K. A. Schat, Antiviral Res., in press).

We thank Colin McLaren for his support and interest during this study and K. Blanchard for secretarial assistance.

LITERATURE CITED


