Thymidine kinase (TK) activity was examined in extracts of TK-deficient cells infected with low passage isolates of herpes simplex virus type 1 (HSV-1) obtained from patients with herpetic keratitis. TK activity induced by the virus, relative phosphorylation rates of thymidine and iododeoxyuridine (IUDR), and Km values for thymidine and for IUDR were compared for TK induced by the different viral isolates. Fourier of the five isolates showing IUDR resistance in vitro and in vivo also exhibited alterations in properties of the viral TK. Two induced very low levels of viral TK. Three (including one with reduced TK) had increased Km values for IUDR. A fifth IUDR-resistant isolate induced TK similar to the IUDR-sensitive isolates. The results indicate that modification of viral gene for TK may be responsible for development of clinical resistance to IUDR in many occurrences of herpetic keratitis. Invest Ophthalmol Vis Sci 27:1546–1548, 1986

Thymidine kinase Activity of Ocular Herpes Simplex Isolates Resistant to IUDR Therapy

Marrho L. Funderburgh,* James L. Funderburgh, and John W. Chandler†

TK activity in cell lysates was assayed at 37°C using 175 μl of the lysate with 75 μl of an assay mix in TK buffer, which provided final concentrations of the following compounds: 2 mM Mg-ATP, 3 mM creatine phosphate, 0.6 mg/ml creatine phosphokinase, and 40 μM thymidine (New England Nuclear, Boston, MA NET-027X) or 40 μM IUDR (Amersham, Arlington Heights, IL TRA.161) each at 20 Ci/mmole. Fifty I samples taken initially, and at three 10-min intervals, were adsorbed onto DEAE cellulose paper circles (DE81-Whatman), and immediately immersed in 90% ethanol. Unphosphorylated nucleoside was removed by three rinses (5 min each) in 90% ethanol, and radioactivity was determined by scintillation...
counting of the dried discs. The rate of phosphorylation of tritiated thymidine was found to be linear for all lysates assayed in this manner. TK activity was calculated from linear regression analysis of the four time points for each assay. TK units were defined as picomoles tritiated thymidine phosphorylated per min. Km values for IUDR and thymidine were determined using the same enzyme extracts described above, diluted twofold in TK buffer. Incubation was carried out as described above in a total volume of 250 μl, containing 10 μl enzyme extract, TK buffer, 2 mM Mg-ATP, 3 mM creatine phosphate, 0.04 mg/ml creatine phosphokinase, and thymidine or IUDR at concentrations ranging from 0.2–19 μM. Hundred microliter samples were removed at 0 min, and after 30 min at 37°C, and phosphorylation was measured as described above. Km values were calculated from Lineweaver-Burke plots.

**Results.** The amount of TK activity in a TK-deficient cell line 14 hr after infection with HSV was found to be a function of the amount of infecting virus added to the culture. Cell lysates from uninfected cultures had no measurable TK. As shown in Figure 1, the amount of TK produced varied among the different viral isolates. Curves similar to those shown in Figure 1 were used to compare the amount of TK induced by eight different ocular viral isolates and one lab strain. These data (Table 1) show considerable variability in the levels of TK induced. Two of the isolates induced levels of TK that were significantly lower than the other seven (P < .01 by t-test). One of these (TFT412) had no detectable activity, and the other (CJ181) induced a TK level 5-50-fold below the other strains. As shown in Table 1, both of these isolates showed resistance to IUDR in vivo and in vitro. Other isolates with IUDR resistance induced TK levels equivalent to or higher than the TK of sensitive viral isolates.

TK induced by the different viral isolates was compared in its ability to utilize thymidine and IUDR as substrates. In the first set of experiments, phosphorylation rates for the two substrates were compared in the presence of high substrate concentrations (40 μM). As shown in Table 2, most of the viral TK from the different isolates utilized IUDR 2–3-fold more rapidly than thymidine. None of the IUDR-resistant isolates was impaired in its ability to phosphorylate IUDR under these conditions. In fact, TK from one resistant isolate (TFT409) phosphorylated IUDR significantly more rapidly than the others at these substrate concentrations.

Usage of the two substrates at lower concentrations was analyzed by the determination of Km values for both thymidine and IUDR in the cell lysates. As shown in Table 2, a significant difference (P < .05) was observed in the Km ratios of three of the isolates. This difference resulted, in each case, from an increase in the Km for IUDR. These TK variants, therefore, require higher concentrations of IUDR to achieve the same phosphorylation rate as average viral TK.

**Discussion.** Of the five viral isolates examined which had demonstrated resistance to IUDR, four showed significant alteration in properties of the TK induced in infected cells. The most dramatic change was the total lack of TK seen in isolate TFT412. As might be expected, this strain was completely resistant to IUDR in vitro. These results support the generally accepted conclusion that viral TK is required for the incorporation of thymidine analogues into viral DNA. Thus, a viral strain can become resistant by the reduction or elimination of its ability to induce TK. The loss of viral TK has been linked with decreased virulence and the inability to establish latency in the trigeminal gan-

![Fig. 1. Relationship between thymidine kinase and an infectious dose of HSV. The amount of TK induced in cultures of TK-deficient B82 cells was determined for several infectious doses of three different HSV-1 isolates using the assay described in Materials and Methods. The lines represent linear regression curves on the original data. Isolates: TFT409 (open circles), E115 (solid circles), CJ181 (squares).](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933355/)
creased phosphorylation of IUDR by these isolates is IUDR in vitro, as well as their clinical resistance to IUDR therapy. The simplest explanation of the decreased phosphorylation of IUDR by these isolates is that it results from an alteration of the viral TK. Other explanations might be possible as well.

In contrast to the other four, one isolate which showed moderate IUDR resistance in vitro appeared to produce normal TK by the criteria of this study. Resistance to IUDR may, therefore, arise independently of alterations in the viral TK. The same conclusion was reached in studies with laboratory strains resistant to another thymidine analogue, acycloguanosine. In conclusion, our data show that alterations in the viral TK may play a role in the development of treatment resistance of HSV to topical IUDR treatment for ocular infections. Alteration of the viral TK, however, may not be the only mechanism by which this resistance can arise.

Table 2. Comparison of thymidine and IUDR as substrates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>$\frac{V_{\text{max}}}{Km}$ (IUDR/Thym)*</th>
<th>$Km$ (gM)</th>
<th>Ratio Km's (IUDR/Thym)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CJ311</td>
<td>1.75</td>
<td>0.50</td>
<td>0.57</td>
</tr>
<tr>
<td>CJ394</td>
<td>1.90</td>
<td>0.20</td>
<td>0.41</td>
</tr>
<tr>
<td>CJ360</td>
<td>2.29</td>
<td>0.56</td>
<td>1.22</td>
</tr>
<tr>
<td>E115</td>
<td>3.20</td>
<td>0.48</td>
<td>1.36</td>
</tr>
<tr>
<td>CJ372</td>
<td>1.66</td>
<td>8.35</td>
<td>1.25</td>
</tr>
<tr>
<td>CJ181</td>
<td>1.43</td>
<td>14.80</td>
<td>1.12</td>
</tr>
<tr>
<td>TFT407</td>
<td>1.13</td>
<td>0.36</td>
<td>0.58</td>
</tr>
<tr>
<td>TFT409</td>
<td>5.48†</td>
<td>1.66</td>
<td>1.34</td>
</tr>
</tbody>
</table>

* Phosphorylation rate in the presence of 40 μM substrate.
† $P < 0.05$ compared to average of four normal ratios (t-test).
‡ $P < 0.01$.

Key words: cornea, herpes simplex virus type 1, 5'-iododeoxyuridine, thymidine kinase, antiviral drugs, drug resistance

References