Cytosine-Arabinoside Does Not Inhibit Incorporation of $^{3}$H-Thymidine in Herpes Simplex Virus Transformed Cells

Brief Report

By

J. ROUBAL

Department of Experimental Virology, Institute of Sera and Vaccines,
Prague, Czechoslovakia

With 2 Figures

Accepted October 25, 1976

Summary

The incorporation of $^{3}$H-thymidine into hamster cells transformed by herpes simplex viruses was found to be resistant to the action of cytosine arabinoside.

In the last few years cells of animal and human origin were transformed in vitro by herpes simplex viruses (HSV) (for references see 17). Several authors demonstrated the presence of virus specific antigens or virus specific nucleic acid sequences in these cells (4, 13) or obtained an indirect evidence on the presence of virus specific functions in them (2, 12).

We have followed the effect of cytosine arabinoside (ara-C), an inhibitor of DNA synthesis, on the incorporation of $^{3}$H-thymidine into the acidprecipitable fractions of hamster cells transformed either spontaneously or by the action of different viruses.

HEF cell line was derived from hamster embryo cultures. Initially the HEF cells exhibited contact inhibition at a saturation density of $10^5$ cells/cm$^2$. At that time, inoculation of $10^6$ cells did not induce tumours in hamsters. After the 100th passage the cells lost contact inhibition and acquired oncogenic potential.

TR-37 cells were derived from HEF cells by transformation with UV-irradiated HSV-2 (14). 333-S-9 and HAM/HSV-1 cell lines transformed by HSV-2 and HSV-1, respectively, in the laboratory of Dr. F. Rapp (Hershey, U.S.A.) were kindly provided by Dr. L. Thiry (Brussels, Belgium). TUB cells were derived in our laboratory from a hamster tumour induced by SV40 transformed H50 cells (1).

Ara-C (Upjohn Co., Michigan) at indicated concentrations was added to the sub-confluent cultures one hour prior to the addition of $^{3}$H-thymidine (20 Ci/mM, Institute for Research, Production and Application of Radioisotopes, Czechoslovakia) at the final concentration of 5 $\mu$Ci/ml. After one hour labelling period
the cells were frozen (−70°C) and thawed. The cell lysis was completed by the addition of concentrated buffer to obtain a final concentration of 0.01 M tris-HCl (pH 7.5), 0.005 M EDTA and 0.2 per cent sarcosylsulphate. The samples were then treated for 18 hours with preincubated pronase (Koch & Light, England) at the final concentration of 1 mg/ml. After addition of the same volume of 10 per cent trichloroacetic acid (TCA) and incubation at 4°C for at least 30 minutes, the precipitates were collected onto Synpor 6 filters (VCHZ Synthesia Uhříněves, Czechoslovakia), washed twice with 5 per cent TCA, once with 96 per cent ethanol and dried. The radioactivity was determined as described previously (10). The rate of 3H-thymidine incorporation in the presence of ara-C was expressed as the percentage of incorporation into the respective cell type in the absence of the drug.

The effect of 20 μg of ara-C/ml is shown in Table 1. As can be seen the incorporation into HEF and TUB cells was strongly inhibited in the presence of ara-C. On the other hand, the incorporation of the label into cells transformed with HSV was resistant to the action of the drug. In fact, more label was incorporated into the treated than into the control cells.

Table 1. Effect of ara-C on 3H-thymidine incorporation into acidoprecipitable fractions of different hamster transformed cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Transforming agent</th>
<th>cpm of 3H thymidine incorporated per culture</th>
<th>Percentage of incorporation in Ara-C presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEF</td>
<td>unknown</td>
<td>Ara-C absent: 9,117 Ara-C present: 418</td>
<td>4.6</td>
</tr>
<tr>
<td>TR-37</td>
<td>HSV-2</td>
<td>10,078 Ara-C absent: 14,099 Ara-C present: 139.9</td>
<td></td>
</tr>
<tr>
<td>333-8-9</td>
<td>HSV-2</td>
<td>6,120 Ara-C absent: 7,345 Ara-C present: 120.0</td>
<td></td>
</tr>
<tr>
<td>HAM/HSV-1</td>
<td>HSV-1</td>
<td>18,132 Ara-C absent: 25,090 Ara-C present: 138.4</td>
<td></td>
</tr>
<tr>
<td>TUB</td>
<td>SV 40</td>
<td>19,896 Ara-C absent: 1,191 Ara-C present: 6.0</td>
<td></td>
</tr>
</tbody>
</table>

As indicated in Figure 1 the incorporation into HEF cells was readily inhibited already at the concentration of 5 μg of ara-C/ml, while the two HSV-2 transformed cell lines incorporated 3H-thymidine still at the concentration of 40 μg of ara-C/ml.

Figure 2 shows that the prolongation of the incubation of the cells with ara-C prior to the addition of the label did not reduce its incorporation into HSV-2 transformed cells.

Several mechanisms can be considered in explaining the resistance of HSV transformed cells to ara-C. At first it was thought that the effect observed might be due to the non-penetrability for the drug induced by membrane changes associated with the transformation by HSV. However, in the subsequent double label experiment the ratios between the amounts of 3H-ara-C (The Radiochemical Centre Amersham, England; 15 Ci/mM; used at the concentration 1.6 × 10−2 μg/ml)