Clinical resistance to acyclovir of herpes simplex virus infections in immunocompromised patients

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Herpes simplex virus strains, isolated from three immunocompromised patients whose infections showed clinical resistance to acyclovir, were studied as treatment progressed. Virus isolated from two patients remained sensitive to acyclovir throughout. Isolates from the third patient, who had received a prolonged course of oral acyclovir, showed a sharp decrease in drug sensitivity which corresponded to loss of thymidine kinase activity. No changes in restriction endonuclease profiles were observed in isolates from the same patient as treatment with acyclovir progressed.

Introduction

Opportunistic viral infections are a common problem in immunocompromised patients with malignant disease. In our study of 156 adult patients with acute leukaemia, non-Hodgkin’s lymphoma or carcinoma of breast or bronchus, 35% of all patients experienced virus infections, 79% of which were with viruses of the herpes group. In one series of patients with acute leukaemia 87% of all virus infections were with herpes simplex virus (Anderson, Sutton & Scarffe, 1984). Such herpes simplex virus (HSV) infections have been treated effectively with acyclovir (e.g., Meyers et al., 1982). However, there is cause for concern in reports of the isolation of resistant strains of HSV from patients undergoing acyclovir therapy (Burns et al., 1982; Crumpacker et al., 1982; Sibrack et al., 1982; McLaren et al., 1983; Wade, McLaren & Meyers, 1983).

We have been monitoring the sensitivity of HSV strains isolated from patients with acute leukaemia and non-Hodgkin’s lymphoma who have been treated with acyclovir by intravenous or oral routes. This brief report concentrates on three patients whose HSV infections showed clinical resistance to acyclovir.

Patients and methods

Patients

The patients described had been admitted to the Medical Oncology service of the Christie Hospital, Manchester, with diagnoses of acute myeloid or lymphoblastic leukaemia. All had received cytotoxic chemotherapy; patient 1 had also been subjected to the severe immnosuppressive regimen associated with bone marrow transplantation. Acyclovir was administered either orally (200 mg qds) or intravenously (5 mg/kg tds).

Virus isolation

Swabs were taken into virus transport medium which was used to inoculate MRC5 human fibroblast and BK tissue cultures. Stocks of virus used in this study were
Table I. Resistance to acyclovir therapy in immunocompromised patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Route of therapy</th>
<th>Serum acyclovir concentration (μM)</th>
<th>ED₅₀ of HSV isolates (μM)</th>
<th>Clinical</th>
<th>In-vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Intravenous</td>
<td>6.45–7.50 (5)</td>
<td>0.053–0.141 (11)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Intravenous</td>
<td>8.40–11.13 (5)</td>
<td>0.035–0.172 (8)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>Oral (+i.v.)</td>
<td>6.00–8.70 (7)</td>
<td>0.092–16.850 (13)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Oral</td>
<td>6.00 (1)</td>
<td>0.277–0.304 (2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Oral</td>
<td>6.15 (1)</td>
<td>0.229–0.246 (2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Oral</td>
<td>6.45 (1)</td>
<td>0.282–0.396 (3)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

a Number of specimens tested; b data from representative patients yielding sensitive strains with full clinical response.

produced by inoculation of Vero cells with HSV isolates at a multiplicity of infection of about 0.01 pfu/cell.

**Plaque reduction assay**

The in-vitro sensitivity of HSV isolates to acyclovir was determined by plaque reduction assay in Vero cells. The method was essentially as described by Field & Darby (1980).

**Thymidine kinase assays**

The thymidine kinase (TK) activities of isolates were determined as described previously (Klemperer et al., 1967; Field, McMillan & Darby, 1981). BU-BHK cells (given by Dr H. J. Field, Cambridge) were used in the assays. These cells are derived from passage of BHK-21 cells in the presence of bromodeoxyuridine, and do not express cellular TK.

**Serum acyclovir levels**

The concentration of acyclovir in selected serum samples, collected for diagnostic purposes without specific relation to the time of acyclovir therapy, was determined using a homogeneous enzyme immunoassay kit (Wellcome Diagnostics).

**Restriction endonuclease analysis**

HSV DNA was extracted from infected Vero cells by a method based on that of Pignatti et al. (1979). DNA samples were incubated for 5 h at 37°C with 5 units/μg of restriction endonuclease EcoRI, Hind III, Kpn I, Hpa I, Bam HI, Xho I, Pvu II, or Bgl II. The DNA samples were analysed by electrophoresis through agarose gels for 18 h at 40 volts, stained with ethidium bromide and viewed with ultra-violet light.

**Results**

**Identity of serial virus isolates**

Restriction endonuclease analysis was carried out on all 21 isolates from patients 1 and 2, using eight enzymes. All isolates were HSV I, and no changes in profile were observed in isolates from the same patient as treatment with acyclovir progressed.
Clinical acyclovir-resistance of herpes simplex

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Sensitivity of isolates to acyclovir

The sensitivities of HSV isolates to acyclovir and the serum acyclovir levels are summarized in Table I. Of the three patients showing clinical resistance (1, 2 and 3), only isolates from patient 1 showed an increase in in-vitro resistance as treatment progressed. The ten resistant isolates recovered from patient 1 after prolonged oral prophylaxis, had a mean ED$_{50}$ value of 10·5 µM acyclovir. This is about 100-fold greater than the ED$_{50}$ of the pre-treatment isolate (0·09 µM) and of the first two isolates recovered after treatment (both 0·12 µM). The development of resistance with time for virus isolated from patient 1 is shown in Figure 1. The resistant virus had no detectable TK activity.

Serum acyclovir levels

The serum acyclovir levels in all patients tested were at least 6 µM, at the time of collection, and would be expected to be effective against sensitive HSV strains. The ED$_{50}$ values for the resistant isolates from patient 1 were higher than the serum acyclovir levels. However, it is difficult to correlate these values with the situation in vivo since the ED$_{50}$ of a virus strain may vary with the cell line or the assay system used (e.g., Harmenberg & Wahren, 1982).
Discussion

Our results show that the acyclovir sensitivity of virus isolates in vitro may not correlate with the clinical response in patients. Isolates from patients 2 and 3 were sensitive in vitro throughout acyclovir treatment, and levels of drug in the serum appeared to be adequate, but the infections responded poorly. Similarly, Wade et al. (1983) described three patients whose lesions remained virus positive despite the isolation of virus strains that were sensitive to acyclovir in vitro. They suggested that this might be due to difficulty with drug penetration, inactivation at the infection site or to characteristics of the individual patients who had severe graft versus host disease and were receiving intensive immunosuppressive therapy.

The influence of virus multiplicity on drug sensitivity may be one explanation for lack of correlation between ‘treatment failures’ and virus sensitivity data in vitro (Harmenberg et al., 1985). Of the four drugs studied by these workers, the response to acyclovir was the most dependent on virus multiplicity.

In the case of patient 1, who had received a prolonged course of oral acyclovir, the clinical resistance of the HSV infection corresponded to loss of viral TK activity, which accounted for the observed decrease in drug sensitivity in vitro. There have been several other reports of the emergence of such TK deficient HSV strains, particularly in immunocompromised patients (see Introduction). It is not clear why this happens. Possibly, in some individuals, insufficient drug penetrates the site of infection, producing conditions of sub-optimal therapy, conducive to the development of resistant strains (see Field, 1982).

This work has been carried out on non plaque purified virus. However, there is evidence that clinical isolates are composed of mixtures of strains with differing drug sensitivities (Field, 1982; Parris & Harrington, 1982). Studies are now in progress on virus clones isolated from patient 1.

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References


