Ganciclovir Is Associated with Low or Undetectable Epstein-Barr Virus DNA Load in Cerebrospinal Fluid of Patients with HIV-Related Primary Central Nervous System Lymphoma

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Background. Epstein-Barr virus (EBV) is pathogenically linked to human immunodeficiency virus (HIV)–related primary central nervous system lymphoma (PCNSL) and is found in virtually all HIV-related PCNSL cases. The objective of this study was to assess the effect of ganciclovir on EBV DNA replication in patients with HIV-related PCNSL.

Patients and methods. EBV DNA was measured by real-time polymerase chain reaction in cerebrospinal fluid and plasma samples from 25 patients with HIV-related PCNSL. Eight of these patients were receiving ganciclovir for concurrent cytomegalovirus infections.

Results. EBV DNA was detected in cerebrospinal fluid samples obtained from 15 (88%) of 17 ganciclovir-untreated patients and 4 (50%) of 8 ganciclovir-treated patients. EBV DNA load was significantly lower for treated patients, compared with untreated patients (median value, 2.15 vs. 4.16 log copies/mL; P = .001). Analysis of sequential cerebrospinal fluid samples from 7 patients showed that EBV DNA decreased in samples obtained from 2 patients following the start of ganciclovir administration but did not decrease in samples obtained from the 5 untreated patients. In addition, patients who received ganciclovir survived longer than the untreated patients (median duration of survival, 181 vs. 72 days; P = .006).

Conclusion. The effect of ganciclovir on EBV DNA load in cerebrospinal fluid supports the hypothesis that EBV is replicating in patients with PCNSL. This observation, together with the effect of ganciclovir therapy on patient survival, suggests that this drug might be useful for the management of PCNSL.

HIV-related primary CNS lymphoma (PCNSL) is associated with Epstein-Barr virus (EBV) infection, which is detected in tumor tissues in almost 100% of cases [1, 2]. EBV DNA is found in the CSF of 90%–100% of patients with PCNSL [2]. Recently, several studies have demonstrated the presence of lytic cycle EBV proteins or transcripts in the tissue and CSF of patients with HIV-related PCNSL. This suggests the possibility of replicative infection in this type of tumor [3–7].

Antiviral agents currently in use, including ganciclovir (GCV), cidofovir, foscarnet, and, to a lesser extent, acyclovir and penciclovir, can inhibit EBV replication [8–11]. In a few instances, antiviral agents have also been used in combination with antiretroviral drugs and/or chemotherapy and radiotherapy for treatment of PCNSL [12–16].

The objective of this study was to assess the antiviral effect of GCV by measuring EBV DNA levels in CSF and plasma samples obtained from patients with HIV-related PCNSL who were treated or untreated with this drug. In addition, the difference in survival between GCV-treated and untreated patients was also evaluated.

MATERIALS AND METHODS

Patients and samples. CSF specimens from 25 HIV-infected patients with PCNSL that had been admitted
Table 1. Demographic and clinical characteristics of patients with primary CNS lymphoma (PCNSL) at the time of diagnosis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GCV-untreated patients</th>
<th>GCV-treated patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 17)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>12:5</td>
<td>5:3</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td>35 (32–41)</td>
<td>35 (30–47)</td>
</tr>
<tr>
<td>Lymphoma, by histological type&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burkitt or Burkitt-like</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Diffuse large B cell</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Not available&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>CD4 cell count, median cells/μL (range)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50 (50–95)</td>
<td>50</td>
</tr>
<tr>
<td>HIV-1 RNA level, median log copies/mL (IQR)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.24 (4.45–5.44)</td>
<td>4.36</td>
</tr>
<tr>
<td>WBC count in CSF, median cells/μL (IQR)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1 (1–4)</td>
<td>1 (1–7)</td>
</tr>
<tr>
<td>Antiretroviral treatment&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Monotherapy or dual therapy</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Ganciclovir treatment&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction&lt;sup&gt;i&lt;/sup&gt;</td>
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<td>2</td>
</tr>
<tr>
<td>Maintenance&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients, unless otherwise indicated. GCV, ganciclovir; IQR, interquartile range.

<sup>a</sup> According to the World Health Organization classification [17].
<sup>b</sup> PCNSL was diagnosed according to clinical criteria.
<sup>c</sup> Data are for 16 GCV-untreated patients and 8 GCV-treated patients.
<sup>d</sup> Data are for 6 GCV-untreated patients and 1 GCV-treated patients.
<sup>e</sup> Data are for 14 GCV-untreated patients and 8 GCV-treated patients.
<sup>f</sup> Following CSF sampling, antiretroviral treatment was stopped for 8 of 12 patients because of worsening of clinical conditions. One patient continued HAART, and 2 patients continued monotherapy. One additional patient started HAART.
<sup>g</sup> Defined as ≥3 antiretroviral drugs.
<sup>h</sup> Following CSF sampling, 6 of these 8 patients continued to receive GCV until death. In addition, GCV therapy was initiated for 4 previously untreated patients.
<sup>i</sup> Induction dose of 5 mg/kg iv was administered twice daily for 6 and 7 days.
<sup>j</sup> Maintenance dose of 5 mg/kg iv was administered daily. Median treatment duration was 335 days (range 227–623) before PCNSL onset.

Follow-up CSF specimens were available from 7 of the 25 patients; these were drawn 20–96 days after first sampling. Two of these 7 patients initiated GCV treatment, 70 and 76 days before the follow-up sampling, after drawing the first CSF sample. Two of the 5 remaining patients discontinued GCV treatment 48 and 19 days before follow-up sampling, and the other 3 were untreated.

Only treatments administered after the first CSF samples were obtained were considered for analysis of survival: 10 patients received GCV (for ≥53 days), 2 received antiretroviral monotherapy (zidovudine or didanosine, for ≥98 days), 2 received HAART ( stavudine, lamivudine, and indinavir, for ≥116 days), 5 received radiotherapy (total dose, 45–50 Gy), and 1 received chemotherapy (2 cycles of methotrexate, administered at a dosage of 1.5 g/m² twice per week every 3 weeks).

Nucleic acid extraction and real-time PCR. Real-time PCR...
was used to measure CSF and plasma EBV DNA levels following nucleic acid extraction, according to a previously described method [19].

**Statistical analysis.** The Mann-Whitney U test was used to compare the EBV DNA load in the different patient groups. Spearman correlation and the χ² test were used to test associations between continuous or categorical variables, respectively. Median values and 25th–75th percentile values are presented for continuous variables. The Cox proportional hazards regression model was used to evaluate the association between survival and continuous variables in a univariate analysis. The Kaplan–Meier method was used to estimate the distribution of survival times. The log-rank test was used for comparisons between survival curves. Negative EBV DNA PCR values were defined for statistical purposes as equal to 2 log copies/mL.

**RESULTS**

EBV DNA was detected in CSF samples from 15 (88%) of 17 GCV-untreated patients and from 4 (50%) of 8 treated patients (p = .028). The viral load was significantly lower in CSF samples from GCV-treated patients, compared with untreated patients (median viral load, 2.15 vs. 4.16 log copies/mL; p = .001) (figure 1). EBV DNA was found in the plasma samples from 1 (20%) of 5 GCV-treated and 3 (33%) of 9 GCV-untreated patients. This represented no significant difference in EBV DNA levels between the 2 groups (median EBV DNA level, 2.00 log copies/mL in both groups). EBV DNA levels in CSF samples did not correlate with plasma levels or CSF cell counts. Twelve patients were receiving ART at the time of CSF sampling. EBV DNA was detected in the CSF samples obtained from 5 (71%) of 7 patients receiving mono or dual ART, 3 (60%) of 5 HAART-treated patients, and 11 (85%) of 13 untreated patients. The levels did not differ significantly between the groups (median EBV DNA level, 3.56 vs. 3.67 log copies/mL).

Among 7 patients with repeated CSF measurements, EBV DNA decreased a median of 2.43 log copies/mL in 2 patients receiving GCV, 1 of whom had also received radiotherapy (total dose, 45 Gy) and HAART (stavudine, lamivudine, and indinavir). The viral load remained stable in 3 GCV-untreated patients (median viral load at baseline, 4.59 log copies/mL; median viral load at follow-up, 4.79 log copies/mL) and increased a median of 3.24 log copies/mL in the 2 patients who discontinued GCV treatment before the second sampling. One of these 5 patients received radiotherapy (total dose, 49 Gy), and another received chemotherapy. Trends did not differ between patients who received and those who did not receive treatments other than GCV with regard to observed EBV DNA levels.

![Figure 1: Epstein-Barr virus (EBV) DNA load in CSF and plasma samples obtained from patients treated with ganciclovir (GCV) and GCV-untreated patients with AIDS-related primary CNS lymphoma. Dotted line, detection limit of the PCR assay; horizontal line, median EBV DNA level.](https://academic.oup.com/cid/article-abstract/42/4/e21/386119)
All but 1 patient died, and the deaths appeared to be related to PCNSL in all of the cases. Overall, the median duration of survival was 85 days (interquartile range, 47–145 days) from the first CSF sampling and 104 days (interquartile range, 72–198 days) from the time of PCNSL onset (i.e., the time when PCNSL lesions appeared in neuroradiological imaging). A number of variables were analyzed with respect to survival time, starting from either the time of CSF sampling or of PCNSL onset. These included EBV DNA levels in the first CSF sample and the use of GCV, any ART, HAART, radiotherapy, or chemotherapy following the first CSF sampling. EBV DNA levels neither correlated with duration of survival as a continuous nor as a dichotomous variable using a cut-off value of 3.51 log copies/mL (the median value of EBV DNA levels in the first CSF sample). No significant correlation was observed between survival time and the receipt of ART, HAART, radiotherapy, or chemotherapy after CSF sampling or lymphoma onset. In contrast, treatment with GCV was significantly associated with a longer duration of survival measured from PCNSL onset ($P < .006$; figure 2).

**DISCUSSION**

We found that treatment with GCV was significantly associated with low or undetectable EBV DNA levels in CSF by applying a real-time PCR technique to the retrospective study of CSF samples obtained from patients with HIV-related PCNSL. Although EBV DNA detection has been used for the clinical management of HIV-related PCNSL for many years, it has only recently been suggested that EBV DNA levels might be influenced by antiviral treatments [6].

Figure 2. Kaplan-Meyer survival curves for patients treated with ganciclovir (GCV) and GCV-untreated patients. Median duration of survival after onset of primary CNS lymphoma was 181 days (interquartile range, 128–251 days) for GCV-treated patients and 72 days (interquartile range, 51–92 days) for GCV-untreated patients. GCV-treated patients received GCV for a median of 272 days (interquartile range, 67–412 days) following onset of lymphoma.

GCV is a nucleoside analogue drug that possesses potent antiviral activity against the lytic phase of EBV in vitro [10]. It is generally agreed that EBV-associated lymphomas express only latent EBV genes, and this theoretically makes antiviral treatment ineffective. However, several studies have shown that viral replicative genes are expressed in tumor cells [3–7, 20, 21]. More recently, lytic cycle mRNA was shown to be expressed in the CSF of patients with brain lymphoma [6], suggesting that these tumors, or at least some tumor cells, might undergo lytic viral replication [3, 7–21].

Although we did not measured mRNA from replicative EBV genes, our findings on the effect of GCV on EBV DNA load in the CSF of patients with HIV-related PCNSL strongly support the hypothesis that EBV replication occurs in this type of tumor. In addition, the observation that CSF EBV DNA load neither correlated with EBV DNA plasma levels nor was associated with high numbers of blood-derived CSF leukocytes is consistent with the intrathecal origin—tumoral origin—of the viral genomes.

There are studies suggesting the usefulness of antiviral agents for the treatment of EBV-related lymphomas [12–22]. GCV and foscarnet have been variably used in association with other treatments (including HAART, chemotherapy, or radiotherapy) in both brain and systemic HIV-related lymphomas and have been associated with clinical remission or prolonged survival time in isolated case reports [12–16]. In the present study, we found that GCV treatment was significantly associated with a longer survival time. Thus, it seems that this drug had some effects on both EBV replication in tumor cells and disease progression. However, it should also be noted that EBV DNA
levels in CSF were not found to directly correlate with survival. This apparent discrepancy might be explained by the fact that some of the patients who were not treated with GCV at the time of sampling received this treatment afterwards.

The introduction of HAART to clinical practice has led to a decrease in PCNSL incidence and to prolonged survival [23–26]. In our study, we observed that HAART had no effect on EBV DNA levels in CSF and no effect on survival. It is possible that the efficacy of HAART was limited among our patients because of their poor viroimmunological response to treatment and the low number of treated patients. On the other hand, it has recently been observed that EBV levels are stable (or even increase) in peripheral blood cells and plasma after HAART initiation [27]. In agreement with our observations, a larger study has recently shown a low overall effect of HAART on survival among patients with PCNSL [28].

In conclusion, treatment with GCV was associated with both reduced EBV DNA load in CSF and prolonged survival among patients with HIV-related PCNSL. This study has the limitation of being based on a small, retrospective, observational case series in which 40% of PCNSL cases were diagnosed according to clinical criteria alone. Nevertheless, these findings might represent a rationale for evaluating, in larger controlled studies, the use of antiviral drugs associated with conventional treatment of HIV-related lymphomas.

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References