Evaluation of Susceptibility of Human Herpesvirus 8 to Antiviral Drugs by Quantitative Real-Time PCR

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A new in vitro system based on real-time PCR was developed for evaluation of human herpesvirus 8 susceptibility to antiviral agents. Cidofovir had the greatest inhibitory activity against HHV-8 (50% inhibitory concentration [IC50], 0.43 μM) followed by ganciclovir (2.61 μM), adefovir (18.00 μM), acyclovir (31.00 μM), and foscarnet (34.15 μM). The potential therapeutic efficacy for HHV-8 (i.e., peak serum drug level/IC50) is highest for cidofovir (167) and foscarnet (22).

The human herpesvirus 8 (HHV-8), also known as Kaposi’s sarcoma-associated herpesvirus, is a new member of the γ-herpesvirinae subfamily that has been associated with human immunodeficiency virus (HIV-1) and non-HIV-related Kaposi’s sarcoma (KS) as well as with multicentric Castleman’s disease and primary effusion lymphoma (5, 6, 17, 25). Despite the decrease in HIV-related KS with the advent of highly active antiretroviral therapy, there is still an interest in evaluating strategies to inhibit the early stages of HHV-8 replication.

Several in vitro systems have been reported for determination of HHV-8 susceptibility to antiviral drugs (10, 16, 20, 22). Because most B cells are latently infected by the virus, inducing agents such as 12-O-tetradecanoylphorbol-13-acetate (TPA) or sodium butyrate have been used to promote lytic viral replication (23). Also, due to the absence of viral plaques in infected lymphoma cell lines, most susceptibility systems were designed to measure viral DNA synthesis. However, the existing assays are very cumbersome, as they require gel electrophoresis and isotopic hybridization. In this report, we describe the development of a rapid and objective in vitro susceptibility system for HHV-8 based on real-time quantitative PCR.

The BCBL-1 cell line, which is a B-cell line latently infected by HHV-8 (23), was kindly provided by Benoît Barbeau (Centre de Recherche en Infectiologie, Ste-Foy, Que´bec, Canada). The BCBL-1 cell line, which is a B-cell line latently infected by HHV-8 (23), was kindly provided by Benoît Barbeau (Centre de Recherche en Infectiologie, Ste-Foy, Que´bec, Canada) using primers designed to amplify the KSHV latency-associated nuclear antigen (LANA) gene (6). The BCBL-1 cell line, which is a B-cell line latently infected by HHV-8 (23), was kindly provided by Benoît Barbeau (Centre de Recherche en Infectiologie, Ste-Foy, Que´bec, Canada) using primers designed to amplify the KSHV latency-associated nuclear antigen (LANA) gene (6). The potential therapeutic efficacy for HHV-8 (i.e., peak serum drug level/IC50) is highest for cidofovir (167) and foscarnet (22).

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FIG. 1. Real-time PCR assay for evaluation of HHV-8 susceptibility to ganciclovir. (A) Amplification plots showing threshold cycle (Ct) values obtained for the HHV-8 ORF26 gene in presence of serial concentrations (from 0 to 32 μM) of ganciclovir. (B) Standard curve of the real-time PCR assay generated using serial 10-fold dilutions of an external HHV-8 standard and from which the Ct values of the different drug concentrations were interpolated. (C) Calculation of the ganciclovir IC₅₀ value based on relative viral load for each drug concentration compared to induced BCBL-1 cells with no drug.
ganciclovir is shown in Fig. 1. Cidofovir had the lowest IC50 value (0.43 ± 0.27 μM), followed by ganciclovir (2.61 ± 1.42 μM), adefovir (18.00 ± 6.36 μM), acyclovir (31.00 ± 9.00 μM), and foscarnet (34.15 ± 1.87 μM) (Table 1). The highest therapeutic ratios (peak serum concentration/IC50 value) were seen for cidofovir (167) and foscarnet (22), whereas adefovir (dipivoxil) had the lowest ratio (0.004) (Table 1).

In this study, we report an innovative and reproducible susceptibility assay for HHV-8 based on real-time PCR using supernatant from TPA-induced BCBL-1 cells. Cidofovir and ganciclovir had the lowest IC50 values, whereas adefovir had intermediate activity and foscarnet and acyclovir exhibited the highest values (Table 1). Noninduced controls expressed very low levels of viral replication, whereas induced controls without drug expressed peak replication levels at 6 to 7 days after TPA induction (data not shown).

Our IC50 values for HHV-8 followed the same trend—although they were not exactly identical—compared to those reported elsewhere using other methodologies (16, 20, 22). For example, although our IC50 values for ganciclovir and cidofovir were virtually identical to those reported by Kedes and Ganem (12), values for foscarnet and acyclovir were approximately half of those determined by the same authors. Such differences may be related to cell type, drug uptake and metabolism, and the methodology used to detect and quantify HHV-8. The greatest inhibitory effect on HHV-8 replication was seen with cidofovir (Table 1). This drug is also the most potent anticytomegalovirus (CMV) drug on the market based on in vitro susceptibility testing (12). Adefovir, which is another acyclic nucleoside phosphate, had notably higher IC50 values against HHV-8. More importantly, if we consider the average peak serum level for each drug after intravenous administration (oral administration in the case of adefovir dipivoxil, which is approved for treatment of chronic hepatitis B infection) (15), the most potent inhibitor is cidofovir, with a peak serum level/IC50 ratio of 167 compared to 22, 12, 5, and 0.004 for foscarnet, ganciclovir, acyclovir, and adefovir, respectively. However, because the primary use of these drugs is likely to be for prophylaxis of KS in high-risk groups or for preemptive therapy at a time of active viral replication but before the development of HHV-8-associated diseases (8, 11, 13, 21, 26), the convenience of administration and the absence of toxicity are two key factors (in addition to IC50 values) that should be considered in the selection of an optimal antiviral agent. In that context, valganciclovir, which is a new bioavailable prodrug of ganciclovir, appears particularly interesting (2, 19).

Accordingly, the protective effect of oral ganciclovir (which is 10-fold less bioavailable than valganciclovir) for the development of KS has been previously reported in a CMV study in HIV-infected subjects (18). However, larger clinical trials using KS as the primary endpoint are needed to confirm this finding.

In conclusion, we designed a new in vitro system for evaluation of HHV-8 susceptibility to antiviral drugs which is more rapid, sensitive, and objective than previous assays based on Southern blot analysis. Similar real-time PCR assays could also be used to assess drug susceptibility of other herpesviruses (24).

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REFERENCES


