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 [IC_{50}], 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/IC_{50}) 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)- 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, Québec, Canada). The cells were maintained as described previously (16). On day 1, 10 ml of BCBL-1 cells at 2 × 10^6 cells/ml in RPMI 1640 medium [Life Technologies, Burlington, Ontario, Canada] supplemented with 10% heat-inactivated fetal bovine serum were pelleted at 250 × g for 10 min and then washed with 2 ml of phosphate-buffered saline. The cell pellet was then resuspended in an equal volume of medium with TPA (without TPA for the negative control) at a final concentration of 20 ng/ml in 25-cm² flasks (BD Biosciences, Oakville, Ontario, Canada). Serial concentrations of antivirals, i.e., acyclovir (zovirax; GlaxoSmithKline), foscarnet (Sigma, Oakville, Ontario, Canada), ganciclovir (cytovene; Hoffman La Roche), cidofovir (vidarabine; Gilead Sciences, Foster City, Calif.), and adefovir (kindly provided by Tomas Cihlar; Gilead Sciences) were made in triplicate and added to culture medium. On day 2, 20 h after TPA stimulation, the cells were pelleted as described above, washed with phosphate-buffered saline, and resuspended in 10 ml of fresh medium containing the same antiviral drugs but without TPA. On day 4 (3 days after the addition of TPA), the same volume of medium containing the same concentrations of antivirals was added to the plates. On the last day (day 7), an aliquot of 1.5 ml of supernatant was removed from the culture for subsequent viral DNA extraction.

The supernatants were centrifuged (2,000 × g for 10 min) followed by treatment with 30 U of RNase-free DNase I for 30 min to remove unencapsidated HHV-8 viral DNA. DNA was extracted from 200 μl of treated supernatant using the QIAamp DNA blood mini kit (QIAGEN, Mississauga, Ontario, Canada) and then eluted in 100 μl of sterile water. The HHV-8 DNA load was determined by a previously described quantitative real-time PCR assay (3) using 5 μl of eluted DNA. Briefly, the competitive real-time PCR assay was performed in a LightCycler instrument (Roche Diagnostics, Laval, Québec, Canada) using primers designed to amplify the orf26 gene (6) and two sets of adjacent fluorogenic probes (one for the target and one for an internal control) to monitor the amplification reaction (3). Fluorescence measurements were performed at each cycle (during annealing) in the F2 (Red-640) and F3 (Red-705) channels, and a threshold cycle (Ct) value for each drug concentration was calculated by determining the point at which the fluorescence exceeded a threshold limit. A standard curve of the Ct values was generated using serial 10-fold dilutions of an external HHV-8 standard from which the C_t values of the different drug concentrations were interpolated. The drug 50% inhibitory concentration (IC_{50}) value was defined as the antiviral concentration that reduced DNA synthesis by 50% compared to TPA-induced controls without drug. Presence of PCR-inhibitory substances was assessed in each sample by verifying that the C_t of the internal control was less than 30.

Under our conditions of TPA stimulation, approximately 1.5 × 10^6 viral genome equivalents were detected in 5 μl of eluted DNA, which corresponds to 3.0 × 10^7 copies per 200 μl of initial cell culture supernatant. A typical susceptibility experiment showing the reduction of HHV-8 DNA synthesis by...
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.
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 foscarnet, ganciclovir, acyclovir, and adefovir, respectively. Thus, it is clear that ganciclovir had the lowest IC₅₀ values, whereas adefovir (dipivoxil) had the lowest ratio (0.004) (Table 1).

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


