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Development and validation of a PCR-based assay for the quantification of Human Cytomegalovirus (HCMV) DNA in samples of frozen whole blood, plasma, urine and semen and the employment of the assay in the development of the HCMV inhibitor 1263W94.

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Background: Compound 1263W94 is a benzimidazole riboside, which demonstrates potent and selective inhibition of HCMV *in vitro*, with an average IC_{50} value of 0.08μM, and is currently in development for the treatment of HCMV disease. Antiviral efficacy may be evaluated by monitoring changes in the levels of HCMV DNA during therapy. Commercially available assays were of limited use as they were insufficiently sensitive, were qualitative, or assayed only plasma samples. We describe here the development of a protocol for the sensitive, reproducible and rapid PCR-based quantification of (HCMV) DNA in samples of whole blood, plasma, semen and urine and the application of the assay in supporting the development of 1263W94. **Methods:** The protocol for a quantitative PCR assay with colorimetric end-point detection was developed from the Roche assay for qualitative detection of HCMV DNA in plasma and used on parallel sample types to evaluate anti-CMV activity of 1263W94 in HIV-infected patients with asymptomatic HCMV shedding (n=54), and in a small number of symptomatic patients (n=7). **Results:** The assay protocol facilitates the quantification of HCMV DNA in samples of semen, frozen whole blood, plasma and urine with a limit of detection of 16 copies/ml and a dynamic range of $>10^3$. The degree of inter- and intra-assay reproducibility is approximately 20% at 10^3 copies/ml. Analysis of samples from HCMV-infected subjects who received 1263W94 revealed a dose-response relationship with a drop in semen HCMV DNA levels of 2.17 $\log_{10}$ copies/ml at a dose of 400 mg tid. A potent antiviral effect was also measurable in blood samples from a second study in which subjects received 1263W94 1200 mg bid or 800mg tid for 8 days where a drop in CMV DNA of > 1 $\log_{10}$ copies/ml was detected. HCMV DNA was never detectable in plasma samples and results from urine samples showed extensive variability. **Conclusion:** We have developed a sensitive and reproducible PCR-based assay, which we have used on several sample types to demonstrate the potent dose-related anti-HCMV activity of 1263W94 *in vivo*.

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Virological, Clinical, and Ophthalmologic Features of Cytomegalovirus Chorioretinitis after Hematopoietic Stem Cell Transplantation.

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Although CMV retinitis is common in patients with AIDS, clinical CMV retinitis is uncommon after hematopoietic stem cell transplant (HSCT), and its risk factors and presentation are poorly defined. We identified 10 patients over a 14-year period that developed CMV retinitis after HSCT. The median day of onset of CMV retinitis after transplant was 251 (range 106-365). Nine of 10 patients had received an allogeneic graft: 7 of whom were seropositive prior to transplant. Clinical extensive chronic graft versus host disease was present in 8 allograft recipients. One additional case occurred in a seropositive autograft recipient. In allogeneic recipients of HSCT who were alive at day 100 after transplant and had chronic clinical extensive graft versus host disease, the incidence was 1.4% (8/577). Five of the 10 patients had other manifestation of CMV disease prior to retinitis (4 gastrointestinal disease, 1 interstitial pneumonia; median time 70 days before onset of CMV retinitis, range 58-279), and 4 others had CMV excretion, thus CMV reactivation anteceded retinitis in 9 of the 10 cases. CMV retinitis was bilateral in 4 patients; 9 of 10 patients had decreased vision and floaters, 1 was asymptomatic. Of 7 patients in whom CMV gB type was determined, 4 had type 1, 2 had type 2 and 1 had a mixed infection by types 1, 2, and 4. Six of 7 patients responded well to ganciclovir or foscarnet systemic treatment, 1 improved only after switching to cidofovir, and 1 patient transplanted in 1983 failed acyclovir treatment. In conclusion, CMV retinitis is an uncommon late complication after HSCT that occurs mainly in seropositive allograft recipients with prior CMV reactivation and chronic GVHD. As long-term survival after HSCT becomes more common, it is likely this complication may be seen more frequently.

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Suppression of constitutive NF-κB activity in cytomegalovirus infected retinal glial cells inhibits virus replication and virus-induced upregulation of interleukin 8

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Infection of different cell types with cytomegalovirus (CMV) results in rapid induction of nuclear factor (NF)-κB. NF-κB binds to the major IE promoter of CMV as well as promoters of different cellular genes whereby inducing their expression. In the present study we observed significance of NF-κB for CMV replication and regulation of cellular genes including intercellular adhesion molecule 1 (ICAM-1) and interleukin 8 (IL-8) expression in human retinal glial cells (HRG). HRG which represent major virus target in patients with CMV retinitis were established from donor eyes. In low-passage cultures infected with CMV (strain AD169) at a multiplicity of infection 2 more than 99% of cells were positive for CMV immediate early (IE) and late (L) antigens. Unlike in other permissive cells such as human fibroblasts CMV did not stimulate NF-κB DNA binding activity in HRG. However, competitive inhibition of the NF-κB nuclear localization sequence of NF-κB p50 with the peptide SN50 (100 μg/ml) resulted in 5-fold reduction of cells expressing CMV IE and L antigens. This treatment had no effects on CMV induced expression of ICAM-1 while upregulation of IL-8 was in part inhibited by SN50 treatment. Clinical inhibitors of CMV replication including ganciclovir or cidofovir had no effects on CMV IE expression or CMV-induced upregulation of cellular genes. The results showed that in retinal glial cells the constitutive NF-κB activity is essential for the CMV-IE expression. Inhibition of the constitutive NF-κB activity by specific NF-κB inhibitors may be important treatment strategy for suppression of virus replication as well as virus induced up-regulation of cellular genes in CMV retinitis.

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Comparison of Drug Susceptibilities of Clinical Isolates of Cytomegalovirus (CMV) for BAY43-9695, BAY38-4766 and Ganciclovir (GCV). J. McSharry and G. Drusano. Albany Medical Coll., Albany, NY 12208, USA.

BAY38-4766 and BAY43-9695 are two non-nucleosidic compounds with antiviral activity against CMV. These compounds block virus replication by inhibiting the cleavage of polygenomic viral DNA. A rapid quantitative flow cytometric drug susceptibility assay and the plaque reduction assay (PRA) were used to determine the drug susceptibilities of 36 CMV clinical isolates to BAY38-4766, BAY43-9695, and ganciclovir. All 36 clinical isolates were susceptible to the two BAY compounds whereas 10 of the 36 clinical isolates were ganciclovir resistant. The average IC_{50} values determined by flow cytometry assay for the drug susceptible CMV clinical isolates for BAY38-4766, BAY43-9695, and GCV are 0.997 ±0.285, 0.887 ±0.317, and 3.186 ±1.734 μM, respectively. The IC_{50} values of the GCV resistant clinical isolates ranged from 17 to 134 μM. The PRA yielded similar results for each of these drugs for these clinical isolates. These results suggest that the two BAY compounds are effective against CMV clinical isolates at drug concentrations that are less than those of GCV. Furthermore, the BAY compounds are effective against CMV clinical isolates that are resistant to GCV. This latter result confirms that the BAY compounds act by a different mode of action than GCV and will be more useful than GCV for the treatment of patients with GCV susceptible and resistant CMV disease.