2005). We now report that CPV is well tolerated in oral toxicology studies in the rat and dog with overall good oral bioavailability. In single dose pharmacokinetic and bioavailability studies, oral bioavailability in rats and dogs ranged from 22 to 46% and 70 to 91%, respectively. In single dose rat toxicology studies, CPV was well tolerated up to 300 mg/kg, with no changes in clinical signs, body weight, organ weights, food consumption, hematology or clinical pathology (NOAEL ≥ 300 mg/kg). Similarly, in a 14-day multiple dose (10–100 mg/kg, PO) rat study, no CPV-related effects on clinical observations, body weight, food consumption, ophthalmic examinations, hematology, or clinical chemistries were observed at any dose tested (NOAEL ≥ 100 mg/kg). CPV was readily absorbed with T_{max} values ranging from 0.50 to 4.0 h on Day 1 and 0.50 to 2.0 h during Week 2. After reaching C_{max}, $t_{1/2}$ elimination values ranged from 1.51 to 3.74 h on Day 1 and 2.37 to 7.26 h during Week 2. The $t_{1/2}$ generally increased with increasing dose and after repeated dosing. Possible sex differences in $t_{1/2}$ and T_{max} were also observed. Toxicokinetic results from the multiple dose rat study show overall CPV exposure increased with increasing dose. Escalating oral doses of CPV (10, 50, 100, and 300 mg/kg) were evaluated in dogs. At doses of 10–100 mg/kg there were no changes in clinical signs, food consumption, body weight or clinical pathology. CPV administration reaching 300 mg/kg resulted in prominent clinical pathology findings observed on Day 12 which correlated with clinical signs and with the kidney identified as a target organ. The MTD for CPV was determined to be 100 and 300 mg/kg for the female and male dogs, respectively. Overall, toxicology studies have demonstrated a very acceptable margin of safety for the advancement of CPV into human phase I clinical studies.

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Phenotyping Human Cytomegalovirus Drug Resistance Mutations Using a Recombinant Virus Incorporating EGFP

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Resistance of human cytomegalovirus (HCMV) to antiviral drugs can be determined either genotypically, by mapping known resistance mutations in genes UL97 and UL54, or phenotypically, by testing viral strains in cell culture in the presence of antiviral drugs. Whereas mutations in UL97 have been well characterised, allowing reliable resistance testing, data about polymerase (UL54) mutations are incomplete. We established a new method for phenotypic characterisation of UL54 mutations. All approved anti-HCMV drugs, Ganciclovir, Cidofovir and Foscarnet, target the viral polymerase. Inhibition of the polymerase leads to reduced synthesis of viral DNA and to reduced expression of late genes. Using a recombinant reporter virus expressing the early late protein pp65 fused to EGFP (vTB65g), we were able to determine drug concentrations required to reduce fluorescence intensity by 50% (IC_{50}). The assay was evaluated regarding MOI dependence, the time of measurement post infection and inter- and intra-test variability and was found to be a highly reproducible, cheap and relatively simple method for the quick analysis of antiviral drug resistance. We were able to determine growth characteristics of vTB65g by generating fluorescence intensity kinetics instead of titrating. In addition, intensity of the pp65-EGFP fluorescence signals strongly correlated with the amount of newly synthesised HCMV genome copies, measured by quantitative realtime PCR, showing that, unlike other assays, our method allows quantitative evaluation of polymerase activity. By combining this assay with powerful markerless BAC mutagenesis, the published phenotype of UL54 mutation E756K was confirmed. To provide the clinically essential link between genotype and resistance phenotype, we generated a database of rules containing all previously published HCMV UL97 and UL54 mutations as well as associated quantitative phenotypic results. Sequence data of UL97 and UL54 from patients' isolates can now be blasted against the database thus allowing a fast screening for drug resistance mutations and corresponding phenotypes. This will provide essential information for an optimum treatment of HCMV-diseased patients.

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The Effect of Human Cytomegalovirus Proteins PUL97 and PUL27 on Host Interferon Responses

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Interferon-gamma (IFN-γ), the sole type II interferon, plays an important role in both innate and adaptive immune responses to viral infection. Human cytomegalovirus (HCMV) alters the expression of both type I and II interferons, but the alteration of IFN-γ signaling by the virus is poorly understood. The viral serine/threonine kinase, pUL97, has been shown to impact multiple host functions as has its putative paralog, pUL27. In murine cytomegalovirus, the M27 homolog of pUL27 has been shown to specifically disrupt IFN-γ signaling. Both pUL97 and pUL27 have been shown to confer resistance to maribavir (MBV) which implied that these viral proteins may have interrelated or redundant functions, so we examined their effect on interferon pathways, specifically under IFN-γ stimulation. MBV is an antiviral that is currently in phase III clinical trials for the treatment of HCMV infections and has been shown to inhibit the kinase activity of UL97. We examined the JAK-STAT and IFN-regulated signaling pathways using real-time reverse transcriptase PCR (RT-PCR) with MBV and a recombinant virus that does not express UL27. The absence of UL27 and/or inhibition of UL97 kinase activity with MBV significantly dysregulates key players within IFN-related signaling pathways, such as IRF-1, ISG15, OAS-1, PDGFRα, MMP-3, IRF-1, SOCS-5 and IP-10 (CXCL10). Upon further evaluation of a subset of statistically significant targets, it was evident that UL97 and/or UL27 may both be involved in the disruption of the host antiviral response via the type II IFN pathway. Investigation of these pathways altered by both pUL27 and pUL97 can clarify the roles of both proteins in viral infection and may improve our understanding of why mutations in both these viral proteins confer resistance to MBV.

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