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Accurate Quantitation of Varicella Zoster virus (VZV) genomic DNA in Infected Rabbit Ocular Tissue using Quantitative Competitive PCR (QC-PCR). EC Dunkel[1], BL Rong[1], Q Zhu[1], M Piatak[2] and D Pavan-Langston[1], From [1]The Schepens Eye Research Institute, and the Department of Ophthalmology, Harvard Medical School, Boston, MA and [2]GeneLabs, Inc., Redwood City, CA, U. S. A.

The rabbit model of VZV-induced ocular and systemic infection has been characterized by clinical disease development, by recovery of infectious virus, by in situ hybridization and by PCR amplification of VZV sequences. The present study extends the PCR analyses to the quantitation of VZV genomic DNA in infected ocular tissues using QC-PCR. Priming sites contained within ORF29 were targeted because this VZV region is highly conserved among a variety of VZV isolates. As a positive control template, and for generation of a competing template, a 697 bp fragment containing the priming region was subcloned into a plasmid vector. The competing template was generated by deleting 75 bp from within the targeted region. Amplification of the wild type template yields a 297 bp product; amplification of the competing template yields a 222 bp product. Increasing copy numbers of competing template are added to replicate aliquots of the positive control or test specimen and amplification performed for 30 cycles. Products were separated on 2% synergel/1% agarose and visualized by ethidium bromide fluorescence under direct UV illumination on a Lynx 4000 video imaging system. After correction for molar equivalence, the integrated fluorescence in the competing PCR product is compared to that in the wild type or unknown derived PCR product. The amount of VZV DNA present in the original test specimen was determined by direct or interpolated assessment of the equivalence point. During acute infection (day 9 PI), VZV copy number ranged from 10^4 to in excess of 10^7/cornea and is dependent upon host responses. The VZV copy number decreased with time after corneal inoculation. A 10 to 1000 fold decrease was demonstrated by QC-PCR in response to antiviral therapy. QC-PCR offers a reliable technique for quantitating VZV DNA in ocular and other tissues. This method will be useful in following the VZV infection from establishment of latency to reactivation in the rabbit model.

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Bovine Herpes Mammillitis Virus Inhibitors in vitro and in a Novel Guinea Pig Vaginal Infection Model. D. F. Smee and J. A. Leonhardt. Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah 84322-5600 USA.

Bovine herpes mammillitis virus or bovine herpesvirus type 2 (BHV-2) causes ulcerative lesions on the teats and udders of cows. We investigated several nucleoside and nucleotide analogs as potential BHV-2 inhibitors, which included acyclovir, ganciclovir, phosphonoformate, 5-iodo-2'-deoxyuridine (IUdR), arabinofuranosyl derivatives of 5-iodocytosine (FIAC), 5-iodouracil (FIAU), and 5-methyluracil (or thymine, FMAU), and various 3-hydroxyphosphonylmethoxypropyl (HPMP) and 2-phosphonylmethoxyethyl (PME) derivatives of adenine (A), guanine (G), 2,6-diamino-purine (DAP), and/or cytosine (C). Of these, FIAU and FMAU were the most potent in cell culture, causing 50% inhibition of BHV-2 plaques at <0.05 μM. HPMPA and HPMPG were active at 0.3 μM; FIAC, IUdR, and HPMPC at 1.3-2.3 μM; PMEDAP and ganciclovir at 20-25 μM; and acyclovir, PMEA, and phosphonoformate at >100 μM. FIAU and FMAU were 50% inhibitory to uninfected embryonic bovine tracheal cell growth at >100 and 53 μM, respectively, giving selectivity indices (ratio of the 50% inhibitory concentration for cell growth to the 50% inhibitory concentration for plaque formation) of >2200 and 1100. Infected cell extracts containing BHV-2 induced thymidine kinase activity phosphorylated FIAU, FMAU, and IUdR at nearly the same rate as thymidine, whereas FIAC, acyclovir, and ganciclovir were not phosphorylated well. In a newly developed BHV-2 vaginal infection model in guinea pigs, FMAU treatments of 1, 3.2, and 10 mg per kg/day for 5 days starting 1 day after virus challenge reduced vaginal lesion scores and virus titers in a dose-dependent manner. These results indicate the potential of antiviral agents to treat bovine herpes mammillitis virus infections in cattle, and the application of a small animal model to study BHV-2 disease. This work was supported by a grant from the Utah Agricultural Experiment Station.