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QC-PCR Accurate Determination of Rabbit Genomic DNA: Evaluation of VZV Load in the Rabbit Ocular Infection Model. Q Zhu[1], M Piatak[2], J Guo[1], S Yamamoto,[1] D Pavan-Langston,[1] and EC Dunkel[1]. [1]Schepens Eye Research Institute; Department of Ophthalmology, Harvard Medical School, Boston, MA and [2]Gene Labs, Inc. Redwood City, CA, USA.

The technique of VZV Quantitative Competitive-PCR (QC-PCR) has been used to analyze and to estimate the number of VZV sequences (copies) retained in ocular tissues from our rabbit ocular VZV infection model. The number of VZV copies detected by QC-PCR however, does not reliably demonstrate the accurate quantitation of VZV load (VZV copy number/number of host cells) in inoculated tissues. This study extends previous QC-PCR VZV quantitation results in the rabbit and develops a QC-PCR approach to quantitation of rabbit genomic DNA. This method allows for accurate determination of the number of rabbit cells involved in the VZV infection. A region in the rabbit phospholamban gene, which is a single copy in rabbit genomic DNA, was selected as our target sequence. A positive control (wild type) plasmid was constructed with a pCR II vector by inserting a 264bp fragment generated from the target sequence. The competitive plasmid (mutant) was developed by deleting 70bp from within the target region. Amplification of the wild type template yields a 264bp product. Amplification of the mutant template yields a 194bp product. Increasing copy numbers of competing template were added to replicate aliquots of the positive control or test specimen and PCR amplification performed for 35 cycles. Products were separated on 2% synergel/1% agarose and visualized by ethidium bromide fluorescence. A standardization reaction using various mutant plasmid concentrations was performed to generate a standard curve. The standardization curve for phospholamban will be used to analyze VZV-infected rabbit tissues to accurately analyze VZV load. The combination of QC-PCR for VZV and QC-PCR for rabbit tissue (host cells) allows us to evaluate not only the VZV load but also to analyze VZV infection dynamics (cell types and number of cells infected with VZV) during VZV acute, latent and reactivated stages in our VZV rabbit model.

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Some Nucleoside Analogs With Anti-Human Immunodeficiency Virus Activity Inhibit Replication of Epstein-Barr Virus

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The effects of (+)-β-D-dioxolane-cytosine [(+)-D-β-DOC], (-)-β-L-dioxolane-cytosine [(-)-L-β-DOC], (+)-β-D-oxathiolane-cytosine [(+)-D-β-OTC], (-)-β-L-oxathiolane-cytosine [(-)-L-β-OTC, or 3TC], 3'-azido-2',3'-dideoxy-5-methyl-cytidine (5-Me-AZDC), and 3'-azido-2',3'-dideoxyuridine (AZDU) on Epstein-Barr virus (EBV) DNA replication in vitro were tested in P3HR-1 cells. 3'-azido-3'-deoxythymidine (AZT) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) were used as positive controls. The inhibitory effects on EBV DNA synthesis were quantified by membrane filter and Southern blot hybridizations with an EBV-specific probe BamHI-W fragment. The 50% effective doses (ED_{50}) for EBV DNA replication were 0.15, 0.83, 1.5, 8.3, 14, and 7.7 μM for DHPG, (-)-L-β-DOC, (+)-D-β-DOC, (+)-D-β-OTC, (-)-L-β-OTC, and AZT, respectively. 5-Me-AZDC and AZDU were not effective at concentrations as high as 30 μM. These results indicated that both (-)-L-β-DOC and (+)-D-β-DOC were more potent than AZT, which has previously shown to have anti-EBV activity. (-)-L-β-DOC and (+)-D-β-DOC have been previously demonstrated to suppress the infectivity of human immunodeficiency virus type 1 (HIV-1). Thus, (-)-L-β-DOC, unlike AZT, represents the first nucleoside analog with L configuration exhibiting antiviral activities for both EBV and HIV. Whether the inhibitory effect of (-)-L-β-DOC is reversible is presently under investigation.