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Optimizing The Detection of Hepatitis B Virus (HBV) Covalently Closed Circular DNA (cccDNA): A Potential Marker For Assessing The Effectiveness of Antivirals. M. Singh, A. Dicaire, M. Krajden, C. Luscombe, and S. L. Sacks, Viridae Clinical Sciences, Inc. and The BC Centre for Disease Control Society, Van., BC, Canada.

Antivirals for HBV reduce viral load and lead to improved liver function with loss of HBe in a proportion of individuals. Persistence of cccDNA and its relative resistance to antivirals is described in animal models but has not been systematically studied in humans. E.g., the role of the immune response against cccDNA in humans during and after therapy of chronic HBV has not been fully explored. cccDNA is formed by converting partially double-stranded (DS) DNA [relaxed circular DNA intermediates (RS DNA)] to fully DS DNA. cccDNA is the major transcriptional template in the nucleus and as such, may prove useful as an indicator treatment efficacy in the future. cccDNA can be differentiated from replicative intermediates by using primers that span the nick in the minus strand. Amplification of RC DNA is inefficient, as the new DNA cannot reach the annealing site of the opposite primer, as long as the RC DNA concentration remains low. Primers used by previous workers are not conserved between genotypes A-F, which could result in false negatives in ethnically diverse populations. Our primers are conserved amongst genotypes A-F and the amplicon is smaller than 300bp. Sensitivity of detection was analyzed using different primer pairs with the Lightcycler system, by using cloned full-length HBV DNA in a plasmid. To differentiate between RC DNA, SS DNA, and cccDNA forms, various enzyme treatments were employed to digest the single strand. Up to 100 copies of cccDNA can be detected using this amplicon-specific hybridization probes. Specificity is maintained at up to 1:1000 ratio of ccc:RC DNA. Our assay allows for rapid and accurate quantification of cccDNA in HBV patients, and will allow us to measure the impact of antiviral treatment on this persistent form of HBV in humans.

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The Recombinant HBV Baculovirus as an Improved Model for the In Vitro Assessment of Antihepadnaviral Compounds. W. Delaney IV[1], D. Colledge[1], R. Edwards[1], J. Torresi[1], R. Chin[1], T. Shaw[1], T.Miller[2], H. Isom[2], T. Bock[3], M. Manns[3], C. Trautwein[3], and S. Locarnini[1]. [1]Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia; [2]Penn State College of Medicine, Hershey, PA, USA; [3]Medizinische Hochschule, Hannover , Germany.

Traditional *in vitro* models for testing anti HBV compounds include the 2.2.15 cell line, transient transfection assays, and primary duck hepatocytes. Although these models have been useful in evaluating anti-HBV compounds, each system has notable drawbacks. In particular, both inter- and intra-experimental variation have been common problems, as has the reproducibility of IC_{50}/IC_{90} data between laboratories. We have previously developed a novel *in vitro* model system for studying HBV replication using recombinant baculoviruses to deliver a 1.3 genome-length HBV *ayw* genome to HepG2 cells (HEPATOLOGY 1998 28: 1134-45). Recently, we have used this system to perform a detailed characterization of the effects of Lamivudine on HBV replication and CCC DNA accumulation *in vitro* (AAC 1999 43: 2017-26). Here, we report the further development of the HBV baculovirus as an *in vitro* model for evaluating antiviral compounds. Using a novel recombinant baculovirus encoding a 1.28 genome length HBV construct (subtype adw_2), we have assayed the inhibitory effects of Lamivudine, PMEA (Adefovir), and Penciclovir on HBV replication in HepG2 cells. Due to greater dynamic ranges of virus expression and large reductions in experimental variability, we observed significant improvements in fitting logistic dose response curves and accurately determining IC_{50} and IC_{90} data. Furthermore, the coefficients of variation for antiviral activity measurements were typically well below 10%. We anticipate that the HBV baculovirus system will be an invaluable tool for further *in vitro* antiviral research including cross-resistance testing of drug resistant HBV strains and evaluating combination therapies.

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Inhibition of the Replication of the DNA Polymerase M550V Mutation Variant of Human Hepatitis B Virus by Adefovir, Tenofovir, L-FMAU, DAPD, Penciclovir and Lobucavir

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Several nucleoside analogues (penciclovir, lobucavir, DXG, DAPD, L-FMAU, lamivudine) and acyclic nucleoside phosphonate analogues (adefovir, tenofovir), that are in clinical use, in clinical trial or under preclinical development for the treatment of HBV infections, were evaluated for their inhibitory effect on the replication of a lamivudine-resistant HBV variant containing the methionine → valine substitution (M550V) in the polymerase nucleoside-binding domain. The antiviral activity was determined in the tetracycline-responsive HepAD38 and HepAD79 cells, which are stably transfected with either a cDNA copy of the wild-type pregenomic RNA or with cDNA containing the M550V mutation. As expected, lamivudine was much less (about 200-fold) effective in inhibiting the replication of the M550V mutant virus than the wild-type virus. In contrast, adefovir, tenofovir, lobucavir, L-FMAU, DXG and DAPD proved equally effective against both viruses. A second objective of this study was to directly compare the antiviral potency of the anti-HBV agents in HepG2 2.2.15 cells (which are routinely used for anti-HBV drug screening purposes) with that in HepAD38 cells. HepAD38 cells produce much larger HBV quantities than HepG2 2.2.15 cells, and thus allow drug screening in multi-well plate format. All compounds proved about equally effective in inhibiting HBV replication in HepAD38 cells as in Hep G2 2.2.15 cells, except for penciclovir which was clearly less effective in HepAD38 cells.

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Establishment Of A Cell Based Assay For Evaluation Of Compounds Against HCV NS2-3 Protease Activity

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HCV polyprotein processing is accomplished by a combination of host and viral proteases. The host proteases are responsible for cleavages at sites between structural proteins and between the structural proteins and NS2. A trypsin-like serine protease encoded in the N-terminal region of the NS3 gene is responsible for cleavage at NS3-4a, NS4a-4b, NS4b-5a and NS5a-5b. The cleavage at the NS2-3 junction involves an additional viral protease which resides within the NS2 and N3 domain. Development of a biochemical assay for NS2-3 protease is hindered by its autocatalytic nature. Here, we reported a cell-based luciferase reporting system for assaying the activity of NS2-3 protease. We observed an induction of luciferase expression when we introduced the wild type construct into cells. Such induction was not seen when the construct carrying a mutation at His952 of NS2 domain was transfected into cells while a similar level of induction was detected when the construct with a mutation at Ser1165 of NS3 domain was used, suggesting strongly that the induction of luciferase expression is NS2-3 cleavage dependent.

To evaluate the system, we tested several commercial available protease inhibitors. We observed the reduction of luciferase expression in a dose –dependent manner with some of the compounds. Inhibition of protease, however, is due to the toxicity of the compounds because a parallel reduction was detected in cultures where induction of luciferase expression was not cleavage-dependent. The only exception is Pepstain A which demonstrated an activity with a therapeutic index of approximately 4. Currently, a stable cell line for this system is under development.