72

Mutations in the Woodchuck Hepatitis B Virus (WHV) Genome that Confer Resistance to Lamivudine and Penciclovir. B. Korba[1], K. Tatti[2], F. Wells[1], J. Gerin[1], and R. Schinazi[2]. 1-Georgetown Univ., DMVI, Rockville, MD USA; 2-Emory Univ., VAMC, Decatur, GA USA.

Single base changes in the sequence of the hepatitis B virus [HBV] polymerase have been shown to confer resistance to lamivudine (3TC) and/or famciclovir/penciclovir [PCV] in both cell culture and in treated patients. Mutations associated with altered drug-sensitivity are located in the binding [B] domain and in the catalytic [C] domain (which contains the conserved YMDD motif). Mutations in the B domain of WHV have been associated with a 3TC-resistant phenotype in treated WHV chronic carrier woodchucks. The predominant change is threonine for alanine at amino acid 565 [A565T]. This mutation is adjacent to a leucine that is in the analogous position to the L526M mutation in the B domain of HBV and is observed in some treated individuals (A527T). Mutations in the YMDD motif have not been observed in treated woodchucks. We constructed three WHV variants: A565T, M588V (analogous to the YVDD mutation in HBV, M550V), and a A565T/M588V double mutant. Following transfection of HepG2 cells, these mutants exhibited drug-sensitivity and replication profiles that paralleled those reported for analogous HBV variants. The M588V mutation was at least 100-fold more resistant to 3TC, but as sensitive to PCV as wild-type. The A565T mutant was approximately 10-fold more resistant 3TC and more than 20-fold more resistant to PCV. The M588V mutant replicated approximately 5-fold less efficiently than wild-type as judged by both extracellular virus production and intracellular DNA replicative forms. The A565T mutant replicated intracellularly as well as wild-type, but because this mutation induces a potential stop codon in the overlapping WHV surface antigen reading frame, extracellular virus production was severely reduced. These experiments further extend the utility of the WHV model to studies of drug resistance. We are currently characterizing the phenotypes of these mutants in adult woodchucks.

73

Viral Dynamics Analysis of HBV Clearance Under Different Antiviral Treatment Regimens Using a Mathematical Model. M. Tsiang[1], G.K.K. Lau[2], L. Cheeseman[1], A. Murray[1], C.S. Gibbs.[1] [1]Gilead Sciences, Foster City, CA. [2]Queen Mary Hospital, Hong Kong

A mathematical model was developed to analyze the dynamics of HBV clearance from patients' serum during antiviral therapy using nucleoside and nucleotide analogues such as lamivudine, famciclovir and adefovir dipivoxil which suppress HBV replication by inhibition of HBV polymerase. This mathematical model includes a provision for incomplete inhibition of viral production described by efficacy ε (where ε = 1.00 for complete inhibition) and accommodates the biphasic clearance of serum HBV DNA observed in patients treated with varying doses of adefovir dipivoxil. The initial rapid phase represents the clearance of free virions present at the initiation of therapy with a half-life of 1.1 days. The 2nd slower phase represents the clearance of virus produced during therapy due to incomplete inhibition and reflects the rate of clearance of infected cells with a half-life of ~18 days. The efficacy of inhibition was 0.800 for 5 mg adefovir dipivoxil once daily and 0.983 & 0.993 for 30 and 60 mg once daily respectively. The effect of increasing efficacy was to increase the magnitude and duration of the initial rapid phase from 0.65 $\log_{10}$ and 4.4 days for 5 mg once daily to ~2.0 $\log_{10}$ and ~10 days for 30 and 60 mg once daily. The effect of nucleoside combination therapy was identical to the effect of increasing efficacy in monotherapy. In a separate study, HBV clearance from patients dosed with 150 mg lamivudine once daily was also biphasic with ε = 0.94 while a combination of 150 mg lamivudine once daily plus 500 mg famciclovir three times daily displayed an increased efficacy of ε = 0.988 and an increased magnitude of the first phase of clearance from 1.1 $\log_{10}$ to 1.9 $\log_{10}$. Simulation of the effects of an induction-maintenance regimen predicted a slight viral load rebound upon dose reduction followed by continued clearance such that the time to elimination is identical to that of the lower dose. This pattern was confirmed in a patient whose dose was reduced from 30 to 10 mg adefovir dipivoxil.