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**WOODCHUCK HEPATITIS VIRUS ANIMAL MODEL FOR ANTIVIRAL  
TREATMENT AND IMMUNOTHERAPY**



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Chronic infection with the hepatitis B virus (HBV) is a major public health problem and is responsible for 1.2 million deaths per year worldwide. Chronic carriers of HBV are at high risk of developing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (primary cancer of the liver). Although safe and effective prophylactic vaccines against HBV are available, antiviral drugs and/or immunotherapeutics to treat chronically infected patients are limited.

The woodchuck hepatitis virus (WHV) and its natural host, the Eastern woodchuck (*Marmota monax*), is a well-characterized mammalian model available for basic and therapeutic research on HBV. The woodchuck model has been useful in studies of the pathogenesis of acute, self-limited



and chronic HBV infection, and in the pre-clinical evaluation of efficacy and, importantly, safety of drug candidates for treatment of chronic HBV infection and for prevention of hepatocellular carcinoma.

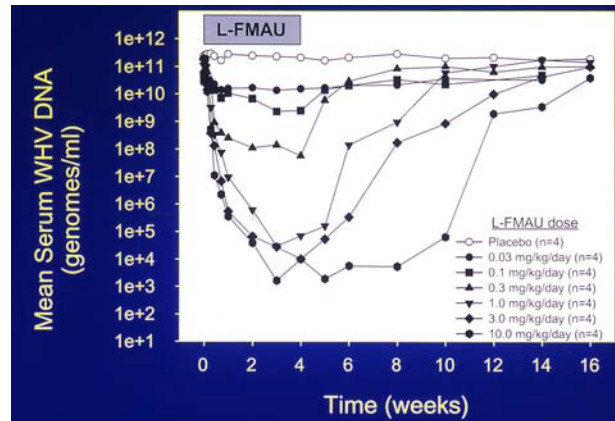


Cornell University currently has a contract from the NIAID, NIH to use the woodchuck animal model for evaluating third generation nucleoside and nucleotide analogs as antiviral drugs, novel immunomodulators for immunotherapy, and new vaccine adjuvants for prophylactic and therapeutic vaccination against chronic HBV infection.

Experimental protocols include efficacy, optimal dosage, pharmacokinetics (PK), safety/toxicity, and combination studies. The following protocols are applied to determine antiviral efficacy:

- 1) **Pharmacokinetics (PK) study.** Many of the antiviral compounds and immunomodulators becoming available for study in the woodchuck model are candidate compounds being developed for human use. For this purpose, a formulation for oral administration is generally indicated although other routes of administration (e.g., intravenous, intraperitoneal, intramuscular, subcutaneous) may be used. The standard approach that has been used to measure pharmacokinetic parameters for a candidate compound in woodchucks involves successive administration of drug intravenously followed after a “wash-out” period by the administration of the drug orally. Multiple blood samples and urine are collected after drug administration and drug concentration measured by the drug developer.
- 2) **Dose-finding study.** Following a PK study and calculation of pharmacokinetic parameters, the effect of antiviral activity of a candidate compound is determined in a dose-finding (dose-ranging) study in chronic WHV carrier woodchucks. Data from such a study provides information for selection of a dose of the candidate compound for more extended antiviral studies including possible combination treatment with other therapeutic agents. The standard approach that has been used to study dose effects in woodchucks involves five groups of five or six woodchucks each that receive different doses of a candidate compound in half log increments over a two-log range for a 4 week period of treatment. With the results, a minimal effective dose can be established and an optimum dose for longer term and/or combination studies estimated. Short-term toxicity also can be evaluated and the dose response relationship established. In addition, it is possible to assess the pharmacodynamic characteristics of a candidate compound by

calculating the half-life of circulating WHV and estimating the synthetic rate of the virus. It is possible to compare the pharmacodynamic effects of equimolar doses of different candidate compounds or of a candidate compound to an antiviral standard, e.g., lamivudine (3TC). Following treatment, woodchucks are monitored for an additional 12 week follow-up period.

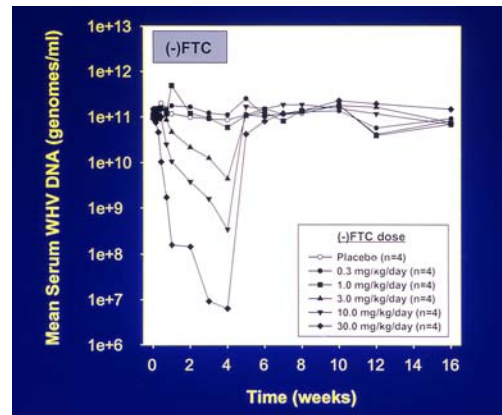


- 3) **Single candidate compound (monotherapy) efficacy study.** Following PK and four-week dose-finding studies, the antiviral efficacy of a candidate compound is assessed during a longer-term study. The dose used for monotherapy of chronic WHV carrier woodchucks will be the one determined to be optimal in the dose-finding study. Treatment with a candidate compound will be performed for 12 weeks, 24 weeks, or longer. Data from such a study provides information on the effect of therapy on disease progression and disease outcome. It also provides long term animal safety data for the drug. In longer-term studies the possible emergence of WHV variants resistant to the candidate compound also can be assessed. The standard approach that has been used to study antiviral efficacy involves groups of chronic WHV carrier woodchucks as small as five or six per group or in life time studies, as large as 20 per group. Treated groups receive doses of the candidate compound daily and controls received vehicle as placebo. An additional group of five or six woodchucks may be included for treatment with a drug of known antiviral activity as a positive control (e.g., lamivudine). Following treatment, woodchucks are monitored for an additional 12 weeks.
  
- 4) **Combination Therapy.** Following a dose-finding study and/or monotherapy study, the antiviral efficacy of a candidate compound in combination with another drug or an antiviral standard such as lamivudine is assessed in a 12-week study. The dose of the candidate compound used for combination therapy of chronic WHV carriers is that determined to be optimal in the dose-finding study and that has a pronounced effect as monotherapy. Combination therapy with a candidate compound may be performed for 4 weeks, 12 weeks, 24 weeks, 36 weeks, or longer. Data from such a study provides valuable information on the effect of combination therapy on disease progression and disease outcome compared to monotherapy. In a longer-term combination therapy study the delay or prevention of resistant WHV variants possibly induced by monotherapy with a candidate compound or an antiviral standard can be assessed. The standard approach that has been used to study combination antiviral efficacy in woodchucks involves

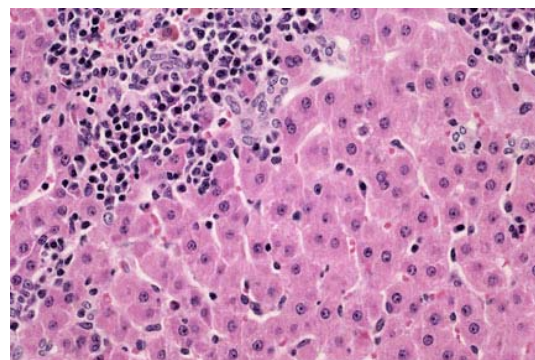
groups of five or six woodchucks that received either the combination of a candidate compound and an antiviral standard (e.g., drug with known antiviral activity such as lamivudine), candidate compound alone, antiviral standard alone, and vehicle as placebo. After cessation of treatment, woodchucks are monitored for additional 12 weeks. Combination therapy studies of a candidate compound with an antiviral standard or with a drug that is licensed for treatment of HBV-infected patients have become increasingly important.

The following assays are used to evaluate efficacy of candidate drugs, immunomodulators, and vaccine adjuvants:

- 1) Slot blot hybridization analysis to assess WHV DNA in serum. Real time PCR is utilized if WHV DNA serum concentration is below the detection limit of the hybridization analysis.
- 2) Immunoassays to measure WHV surface antigen (WHsAg) and antibodies against WHsAg (anti-WHs antibody) and WHV core antigen (anti-WHc antibody) in serum.



- 3) Southern blot hybridization analysis to determine WHV DNA in liver, with differentiation between WHV DNA monomers (replicative WHV DNA intermediates) and covalently-closed circular WHV DNA (WHV cccDNA).
- 4) Northern blot hybridization analysis to measure WHV RNA in liver.
- 5) Histological examination of liver tissues to assess hepatic inflammation (CD3+ cells), proliferative response of hepatocytes (proliferating cell nuclear antigen; PCNA), and apoptosis in hepatocytes (terminal deoxynucleotidyl transferase-mediated uridine deoxynucleotide nick end labeling (TUNEL) assay). The histological alterations in tissues examined include portal hepatitis, lobular hepatitis, bile duct proliferation, steatosis, and liver cell dysplasia.



- 6) Immunohistochemical examination of liver tissues to determine expression of hepatic WHcAg and WHsAg.

- 7) Automated chemistry panels and complete blood counts (CBCs) are used to evaluate overall health and possible drug toxicity. Hepatic cell injury in woodchucks as a consequence of chronic WHV infection and possible toxicity associated with treatment is evaluated using changes in the serum enzyme activities of  $\gamma$ -glutamyltransferase (GGT), sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Cholestatic injury is assessed by measuring alkaline phosphatase (ALP) and serum bilirubin. Possible kidney injury and altered glomerular function are assessed by measuring blood urea nitrogen and creatinine. Bone marrow depression and/or hemolytic anemia are evaluated via complete blood counts. Electrolyte and acid-base status is assessed by measuring Na, K, Cl, Ca,  $P_i$ ,  $HCO_3^-$ , and anion gap.
- 8) In vitro proliferation assay of peripheral blood mononuclear cells (PBMCs) to measure woodchuck T cell responses to WHV core antigen (WHcAg) and WHsAg. Synthetic peptides of both antigens are used to determine epitopes recognized by T cells.
- 9) Real-time RT-PCR-based assay to determine mRNA expression levels of TH1- and TH2-type cytokines (IL-2, IL-4, IL-6, IL-10, IL-12, IFN- $\alpha$ , IFN- $\gamma$ , and TNF- $\alpha$ ) and leukocyte surface markers (CD3, CD4, CD8) in woodchuck PBMCs and liver.

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