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Suppression of lamivudine-resistant B-domain mutants by adefovir dipivoxil in the woodchuck hepatitis virus model

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Abstract

Adult woodchucks (*Marmota monax*) chronically infected with woodchuck hepatitis virus (WHV) were treated orally with lamivudine (15 mg/kg per day) for 57 weeks. After 20 weeks of treatment a 2–3 log reduction in serum WHV DNA was detected. Serum titers of WHV then increased gradually, in the presence of lamivudine treatment, reaching pre-treatment values by week 40. Viral recrudescence was associated with development of mutations in the B domain of the WHV polymerase gene. Mutations observed in the highly conserved FLLA motif of the B domain were L564V, L565M, and A566T, with A566T being the most frequently observed. Beginning on week 57 of lamivudine treatment, one group ($n = 3$) was treated orally with adefovir dipivoxil at a dose of 15 mg/kg per day plus lamivudine, and a second group ($n = 3$) was treated with H_2O placebo plus lamivudine. In woodchucks treated with adefovir dipivoxil, two had the A566T mutation, and one had both A566T and L565V. In the group maintained on lamivudine monotherapy, A566T alone was present in one animal, another carried both A566T and L565V, and in the third, no B-domain mutations were detected. There was a 4.5 log reduction in serum WHV DNA after 12 weeks of treatment with the adefovir/lamivudine combination, while in the lamivudine monotherapy controls, WHV DNA decreased by only 0.83 log ($P > 0.001$). A slight recurrence in serum titers of WHV DNA was observed one week after withdrawal of adefovir treatment but no further increase in viral load was observed during the remainder of the 12-week post-treatment follow-up period. The results demonstrate that supplemental adefovir dipivoxil treatment is effective in suppressing replication of lamivudine-resistant B-domain mutants in the woodchuck model of hepatitis B virus infection.

Keywords: Woodchuck hepatitis virus; Drug-resistance; Antiviral therapy; Adefovir dipivoxil; Lamivudine

1. Introduction

Over 350 million people worldwide are chronic carriers of hepatitis B virus (HBV). Chronic viral hepatitis infections can progress to cirrhosis, which may ultimately lead to hepatic failure or hepatocellular carcinoma (Hoofnagle and Di Bisceglie, 1997). Long-term monotherapy with the nucleoside analog lamivudine for chronic HBV infections often results in the emergence of lamivudine-resistant HBV mutants (Hussain and Lok, 1999; Dienstag et al., 1999; Lok et al., 2001; Oh et al., 2002). Adefovir dipivoxil (ADV) has been shown to be effective in treatment of such HBV mutants (Xiong et al., 1998; Perrillo et al., 2000), and was recently approved by the Food and Drug Association for the treatment of chronic hepatitis B (Anon, 2002). Treatment with adefovir dipivoxil for 48 weeks did not result in emergence of adefovir resistant mutants during phase III studies (Westland et al., 2003; Marcellin et al., 2003; Hadziyannis et al., 2003). Treatment with ADV has been shown to result in the infrequent emergence of resistance mutations in the HBV polymerase D domain, but these virus remain sensitive to lamivudine (Angus et al., 2003).

The woodchuck model has been useful in studies of the pathogenesis of chronic HBV infection (Tennant and Gerin, 2001). The genomic structure of the woodchuck hepatitis virus (WHV) is similar to that of HBV, and the biological properties, including the replication strategies of the two viruses, are similar (Seeger and Mason, 2000). Both viruses induce chronic infections, both are associated with chronic hepatitis and hepatocellular carcinoma, and both have the capacity to integrate into host cell DNA.

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Chronic WHV carrier woodchucks have been useful in the preclinical evaluation of antiviral compounds for treatment of chronic HBV infection. Numerous antiviral agents have characteristically demonstrated efficacy in WHV chronic carriers similar to that observed in HBV-infected patients (see Korba et al., 2000a; Peek et al., 2001 for reviews). ADV has been shown to be safe and effective as an antiviral agent in chronic WHV carrier woodchucks (Cullen et al., 2001). During previous long-term studies of lamivudine therapy in chronic WHV carriers, resistance of WHV to therapy was associated with the appearance of mutations in the WHV polymerase gene (WHVpol). The most common mutation, A566T, is located in the FLLA motif of the B domain (Mason et al., 1998; Tatti et al., 2002; Zhou et al., 1999). The predominant clinical mutations associated with lamivudine-resistance in man are in the HBV polymerase C domain (YMDD motif) (M204V/I), however the B-domain (FLLA motif) mutations (L180M, A181T) are also capable of causing biological reduction in lamivudine susceptibility in both species of virus (Ciancio et al., 2004; Ono et al., 2001; Yeh et al., 2000; Fu and Cheng, 1998). The phenotypic properties of WHV carrying WHVpol B-domain mutations in cell culture studies parallels those observed for the L180M mutation in the homologous B domain of HBV, and the more infrequent HBV A181T mutation (Yeh et al., 2000), that have been associated with clinical lamivudine and famciclovir resistance (Allen et al., 1998; Aye et al., 1997; Delaney et al., 2001; Ling et al., 1996; Tatti et al., 2002; Xiong et al., 2000). The objective of this study was to test the in vivo efficacy of ADV in woodchucks with lamivudine-resistant, chronic WHV infection.

2. Materials and methods

2.1. Experimental animals

Woodchucks were maintained in a controlled laboratory environment at Cornell University, Ithaca, NY, USA. All experimental procedures involving animals were performed under protocols approved by the Cornell University Institutional Animal Care and Use Committee. The woodchucks used in this study were born in the laboratory-maintained breeding colony to WHV-negative dams in February through April 1998 and 1999. Woodchucks were inoculated at 3 days of age with a WHV inoculum derived from a cloned virus (cWHV8; 5×10^6 WID_{50}) (Cote et al., 2000). At 1 year of age, woodchucks were anesthetized (ketamine 50 mg/kg and xylazine 5 mg/kg IM), blood samples obtained, and the presence of woodchuck hepatitis virus surface antigen (WHsAg) in serum was verified by enzyme immunoassay (EIA, 1:10–1:4000 dilution) using purified 22-nm diameter WHsAg as standard (Cote et al., 1993).

2.2. Experimental design

Thirteen WHV carrier woodchucks were treated initially with lamivudine to induce the emergence of lamivudine-resistant WHV mutants. Lamivudine (15 mg/kg per day) was administered once daily, orally, by dose syringe with 1–2 ml of semi-purified liquid diet (Dyets Inc., Bethlehem, PA, USA) to ensure consumption (Peek et al., 2001). Woodchucks were bled weekly for the first 4 weeks, at weeks 6 and 8, and thereafter at 4-week intervals. After 44 weeks of continual lamivudine treatment, serum WHV was analyzed for the presence of mutations associated with lamivudine-resistance by direct sequencing of PCR amplification products (Tatti et al., 2002; MWG Biotech, Inc., High Point, NC, USA). Due to the prolonged treatment interval, several animals developed markers of hepatocellular carcinoma (HCC), a natural consequence of long-term chronic WHV infection (Tennant and Gerin, 2001), and were therefore not suitable for secondary therapy with ADV.

2.3. Combination therapy

Six woodchucks in which viral recrudescence had occurred were selected to assess the effect of the addition of ADV therapy to current lamivudine treatment (combination therapy). Animals were stratified based on the presence of high, stable serum titers of WHV, and WHVpol B-domain mutants. Three animals with WHVpol B-domain mutants received 15 mg/kg per day ADV in addition to 15 mg/kg per day lamivudine, and three (two with WHVpol B-domain mutants and one with only wild-type WHV detectable) received H_2O as placebo in addition to 15 mg/kg per day lamivudine as controls (lamivudine monotherapy). All doses were administered orally by dose syringe as described above. Both groups were treated for 12 weeks and observed for an additional 12 weeks post-treatment during which lamivudine monotherapy was continued in both groups. The woodchucks were bled weekly for the first 4 weeks during combination therapy and thereafter at 2-week intervals during the remainder of the study. At the end of the post-treatment follow-up, woodchucks were euthanized and postmortem examinations were performed.

2.4. Measurement of virologic markers

Viral load, expressed as WHV DNA genome equivalents/ml (WHVge/ml), was measured in serum samples collected during the study by slot blot assay using 10 μl of serum compared with a standard dilution series of the pWHV8 (assay cut off 0.005 ng/10 μl = 1.6×10^8 WHVge/ml) (Cote et al., 2000). Intra-assay variation was controlled by repeating sequential serum samples from previous time points. A drop in viral titers observed between weeks 52 and 56 may be accounted for by the manner in which the serum samples were stored prior to assay. Serum collected during weeks 0–52 were stored at −70 °C

prior slot blot analysis. Serum samples collected during the combination treatment and withdraw period (weeks 56–81) were processed immediately after short-term storage at −20 °C. When levels of serum WHV DNA were below the detection limits of the slot blot assay, WHV DNA was extracted from 200 μl of serum using commercial reagents and the manufacturer's recommended procedures (Qiagen, Valencia, CA, USA). WHV DNA was quantified by real time polymerase chain reaction (PCR) using previously validated forward and reverse primer sets and probe (Taqman System; Applied Biosystems, Foster City, CA, USA) (Cote et al., 2000). The sensitivity of the assay was 6000 WHV genome equivalents per reaction.

2.5. Statistics

The geometric means of the serum WHV DNA values for each group of three woodchucks were calculated. Student's *t*-test for two samples of unequal variance was used for statistical comparison between groups at each sampling date. In this analysis, the differences between the pre-treatment value and the value at each sampling date during and after combination therapy were calculated for each group, and the values of each group compared.

3. Results

3.1. Initial lamivudine monotherapy

In the six woodchucks that received lamivudine monotherapy and selected for the subsequent treatment study, the geometric mean serum level of WHV declined from 1.92×10^{11} WHVge/ml before initiation of lamivudine monotherapy to 2.29×10^{9} WHVge/ml after 20 weeks of monotherapy (Fig. 1). This reduction in viral load was maintained for an additional 8 weeks after which time the viral load gradually increased. By week 40, the viral load levels approached those observed pre-treatment.

Table 1
Mutations within the polymerase gene of WHV from carrier woodchucks treated with lamivudine (3TC) for 44 weeks

Treatment	Animal number	Mutation	
		B domain	C domain
Lamivudine + placebo	4490	Wild type	Wild type
	5681	A566T	Wild type
	5649	L564V + A566T	Wild type
Lamivudine + adefovir dipivoxil	4481	A566T	Wild type
	5675	A566T	Wild type
	4491	L564V + A566T	Wild type

Molecular analysis of serum WHV DNA after 44 weeks of treatment demonstrated single or double base mutations within the WHV polymerase B domain. The mutant status of the woodchucks that received either ADV/lamivudine combination or were continued on lamivudine monotherapy are shown in Table 1. In three of the woodchucks, the A566T mutation was detected alone (one used for monotherapy, two for combination therapy). In two, A566T was present with a second B-domain mutant, L564V (Table 1) (one used for each treatment regimen), and in the sixth woodchuck no mutations in the B domain were detected (used for monotherapy). No mutations were detected in the WHV polymerase C domain, particularly in the YMDD motif. All 5 animals carrying WHV mutants as the dominant circulating virus also harbored WHV containing wild-type sequences in the

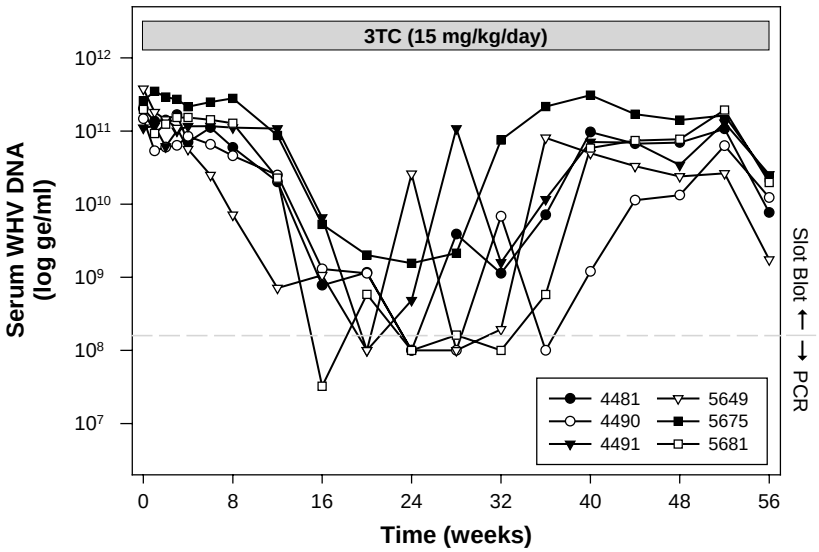

Fig. 1. Serum WHV DNA levels of woodchucks with chronic WHV infection treated orally with lamivudine (3TC; 15 mg/kg per day) monotherapy for 57 weeks.

Table 2
Change from baseline in geometric mean WHV DNA levels during and after combination treatment with adefovir dipivoxil (ADV) plus lamivudine ($n = 3$) or placebo plus lamivudine (3TC) ($n = 3$)

Treatment group	Pre-treatment week 57	Treatment week					Week post-treatment				
	Lamivudine monotherapy	1	2	4	8	12	1	2	4	8	12
ADV + 3TC											
Geomean ($\log_{10}$ ge/ml)	10.29	9.62	9.59	9.35	8.10	5.78	7.08	6.86	6.27	5.90	7.07
log Δ	–	−0.67	−0.7	−0.94	−2.19	−4.51	−3.21	−3.43	−4.02	−4.39	−3.22
Placebo + 3TC											
Geomean ($\log_{10}$ ge/ml)	9.76	9.74	9.73	9.59	9.26	8.93	8.10	7.9	6.67	5.85	6.81
log Δ	–	−0.02	−0.03	−0.19	−0.5	−0.83	−1.66	−1.86	−3.09	−3.91	−2.95
P-value[a]		0.024	0.25	0.072	0.045	0.0003	0.13	0.12	0.34	0.46	0.7

[a] Comparison of log changes from pre-treatment between geometric mean WHV levels in ADV + 3TC group vs. Placebo + 3TC group.

B domain as a minor species (approximately 10–20% of the total circulating virus), similar to that observed in earlier studies with long-term lamivudine monotherapy (Tatti et al., 2002). No changes in the WHVpol B or C domain sequence patterns were observed after either 4 or 12 weeks of ADV treatment.

3.2. Combination therapy

After 57 weeks of lamivudine monotherapy, the geometric mean viral load in the woodchucks selected to receive the adefovir combination therapy was 1.95×10^{10} and 5.75×10^{9} WHVge/ml in the lamivudine monotherapy group (Table 2, Fig. 2). After 8 weeks of treatment, the mean viral loads had decreased by 2.19 log in the combination therapy group, whereas it had decreased by only 0.5 log in the lamivudine monotherapy group ($P = 0.045$). By week 12, the mean viral load had decreased by 4.51 log in the ADV treated group, compared to a 0.83 log reduction in the monotherapy control group ($P = 0.0003$).

Upon withdrawal of ADV treatment, mean viremia levels transiently increased approximately 1 log, and then gradually declined to a lower, stable level. Due to a continued decline in viremia in the lamivudine monotherapy group, the difference between the two treatment groups during post-treatment follow-up was no longer statistically significant (Table 2, Fig. 2).

Fig. 3 expresses the viral load of the ADV group as a fraction of the placebo lamivudine monotherapy control group, summarizing the efficacy of ADV therapy in animals harboring WHVpol B-domain mutants. No changes in the sequence profiles of the dominant circulating virus were observed in any of the animals during ADV treatment or the subsequent follow-up periods (data not shown).

No clinical evidence of toxicity was observed during the period of ADV treatment and no pathologic changes attributable to ADV treatment were observed during post-mortem examinations performed at the end of the study.

4. Discussion

The nucleotide analog adefovir dipivoxil (ADV) has been shown to be effective in the treatment of HBV carriers infected with lamivudine-resistant HBV carrying mutations in the YMDD motif of the C domain of HBVpol and in the FLLA motif of the HBVpol B domain (Liaw et al., 1999; Ono-Nita et al., 1999; Yang et al., 2002; Hadziyannis et al., 2003; Marcellin et al., 2003; Perrillo et al., 2000). In this study, we have demonstrated that lamivudine-resistant WHV carrying mutations in the FLLA motif of the WHVpol B domain are sensitive to ADV in chronically infected woodchucks. In woodchucks, lamivudine resistance in WHV is associated exclusively with mutations of the WHVpol B domain that are similar to the FLLA domain mutations in HBV (Mason et al., 1998; Tatti et al., 2002; Zhou et al., 1999). In cell culture, WHV with mutations of the FLLA motif, as well as WHV carrying engineered mutations in the YMDD motif of the WHVpol, are viable and exhibit the same patterns of sensitivity/resistance to antiviral agents as are observed for HBV in vivo and in vitro (Tatti et al., 2002). In our study, A566T mutation was present alone and in combination with two other FLLA motif mutations (L564V, L565M). The L564V mutation has not been described previously.

The reason for the reduction of viral load in the groups after treatment withdraws in this study can be explained. This antiviral trial was longer than most antiviral studies since the woodchucks were first treated with lamivudine for more than 1 year to select for lamivudine-resistant mutants. WHV carriers have been observed at the end of life to have spontaneous reductions in viral load associated with increasing hepatic tumor burden (Tennant and Gerin, 2001). At least five of the six animals used for the ADV treatment study carried serologic markers of HCC (e.g., elevated GGT; Tennant and Gerin, 2001) by the end of the subsequent ADV treatment period. Specifically, tumors were detected in woodchuck 5649 (placebo control group) on week 46 of this study, which appeared to suppress viral loads. Whereas, tumors detected in woodchuck 5675 (ADV treatment group) on week 58 of this study led to the death of the animal. In a previous

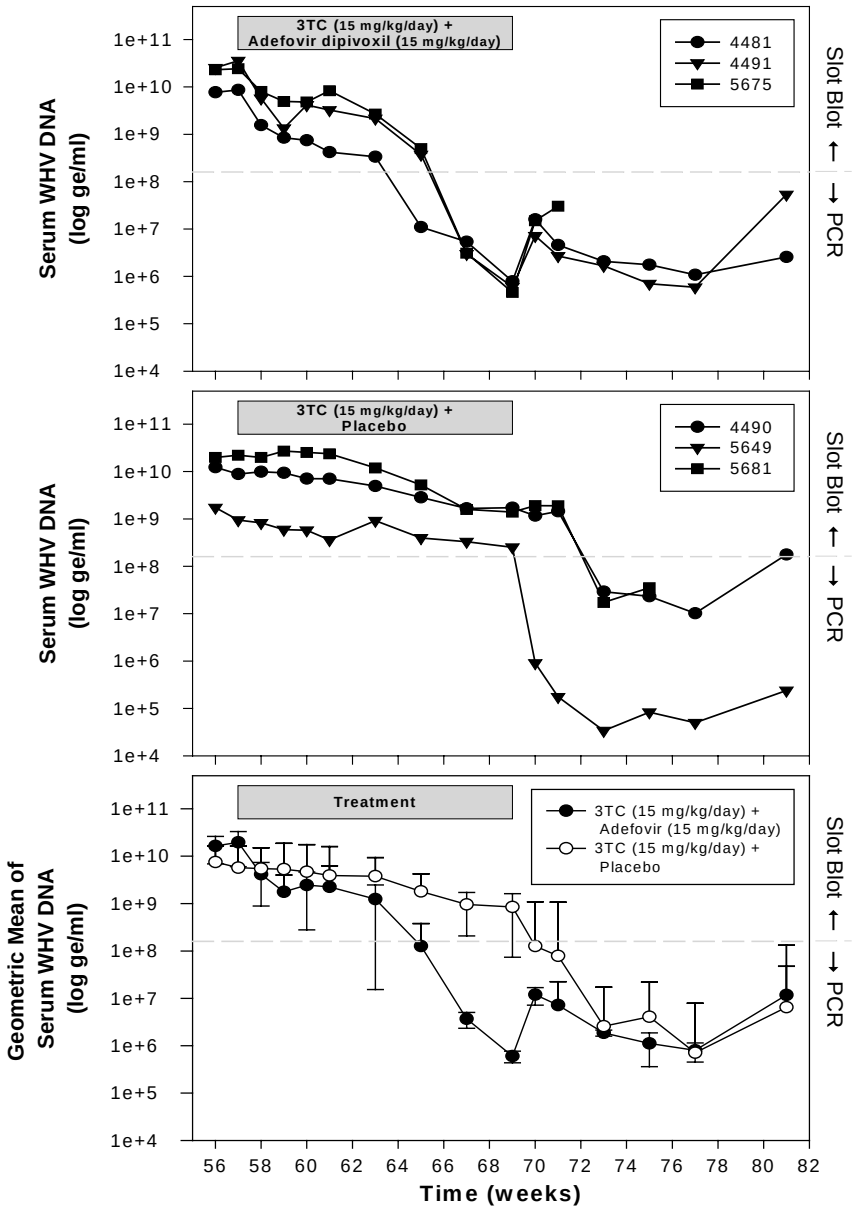

Fig. 2. Serum WHV DNA levels of woodchucks with chronic WHV infection treated orally with lamivudine (3TC; 15 mg/kg per day) monotherapy for the duration of the 81-week study. Beginning on week 57 three of the six animals were administered adefovir dipivoxil 15 mg/kg per day (top panel) and the remaining three animals were given H_2O placebo (middle panel) for 12 weeks (weeks 57–69). Lamivudine therapy continued in both groups after withdrawal of combination treatment until the end of study (weeks 69–81).

study of long-term lamivudine monotherapy in woodchucks, similar reductions in viremia were observed following a period of viral recrudescence that was linked to the emergence of WHV with A566T mutations (Tatti et al., 2002). In light of these issues, and the experimental design of this study, the difference between treatment regimens was still significant.

WHV in one of the animals in this study exhibiting recrudescence of viremia under lamivudine therapy did not appear to carry mutations in either the WHVpol B or C domains. Several studies have reported a similar lack of ability to detect mutations in the HBVpol B or C domains in an average of 25% (15–50%) of patients experiencing rebounds in HBV titers while under lamivudine therapy (Ciancio et al., 2004). No underlying mechanism for these observations has been defined.

The current study, as well as previous studies with lamivudine/famciclovir (Korba et al., 2000b), lamivudine/alpha interferon (Korba et al., 2000c), and clevudine/WHsAg vaccine (Menne et al., 2002) demonstrate the utility of the WHV/woodchuck model in providing experimental evidence to support the use of combination therapies for chronic HBV infection. The results of this study are similar to those observed in clinical trials with ADV therapy of

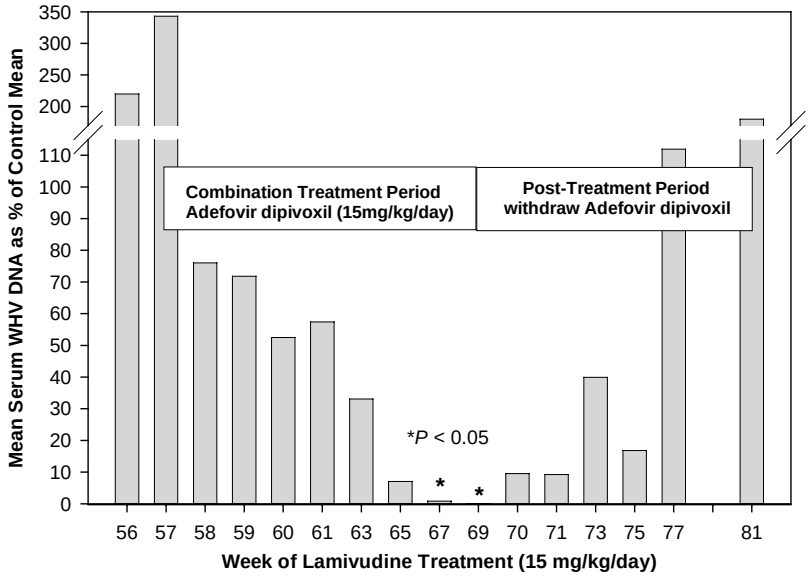

Fig. 3. Reduction in serum WHV DNA of three woodchucks with lamivudine resistant, chronic WHV infection during 81 weeks of lamivudine treatment (15 mg/kg per day) treated in combination with adefovir dipivoxil (15 mg/kg per day) for 12 weeks (weeks 57–69), then monitored for 12 weeks after withdrawal of treatment (weeks 69–81). Serum WHV DNA of the drug treated group is expressed as a percentage of the placebo control values.

patients carrying lamivudine-resistant HBV infections when given either as a monotherapy, or as a combination therapy (Xiong et al., 2003; Perrillo et al., 2000; Peters et al., 2004; Perrillo et al., 2004). Although the lamivudine resistance mutations were slightly different in WHV the response of lamivudine-resistant WHV mutants to ADV in woodchucks is consistent with cell culture studies demonstrating the susceptibility of lamivudine vested HBV to ADV (Xiong et al., 1998) and extends to the utility of the woodchuck model to in vivo studies of drug-resistance.

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References

Allen, M.I., Deslauriers, M., Andrews, C.W., Tipples, G.A., Walters, K.A., Tyrrell, D.L., Brown, N., Condreay, L.D., 1998. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 27, 1670–1677.

Angus, P., Vaughan, R., Xiong, S., Yang, H., Delaney, W., Gibbs, C., Brosgart, C., Colledge, D., Edwards, R., Ayres, A., Bartholomeusz, A., Locarnini, S., 2003. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 125, 292–297.

Anon., 2002. New treatment approved for chronic hepatitis B. FDA Consum. 36(6), 4.

Aye, T.T., Bartholomeusz, A., Shaw, T., Bowden, S., Breschkin, A., McMillan, J., Angus, P., Locarnini, S., 1997. Hepatitis B virus polymerase mutations during antiviral therapy in a patient following liver transplantation. J. Hepatol. 26, 1148–1153.

Ciancio, A., Smedile, A., Rizzetto, M., Lagget, M., Gerin, J., Korba, B., 2004. Identification of HBV DNA sequences that are predictive of response to lamivudine therapy. Hepatology 39, 64–73.

Cote, P.J., Korba, B.E., Miller, R.H., Jacob, J.R., Baldwin, B.H., Hornbuckle, W.E., Purchell, R.H., Tennant, B.C., Gerin, J.L., 2000. Effects of age and viral determinants on chronicity as an outcome of experimental woodchuck hepatitis virus infection. Hepatology 31, 190–200.

Cote, P.J., Roneker, C., Cass, K., Schodel, F., Peterson, D., Tennant, B.C., deNoronha, F., Gerin, J.L., 1993. New enzyme immunoassays for the serologic detection of woodchuck hepatitis virus infection. Viral Immunol. 6, 161–169.

Cullen, J., Li, D.H., Brown, C., Eisenberg, E.J., Cundy, K.C., Wolfe, J., Toole, J., Gibbs, C., 2001. Antiviral efficacy and pharmacokinetics of oral adefovir dipivoxil in chronically woodchuck hepatitis virus-infected woodchucks. Antimicrob. Agents Chemother. 45, 2740–2745.

Delaney IV, W.E., Edwards, R., Colledge, D., Shaw, T., Torresi, J., Miller, T.G., Isom, H.C., Bock, C.T., Manns, M.P., Trautwein, C., Locarnini, S., 2001. Cross-resistance testing of antihepadnaviral compounds using novel recombinant baculoviruses which encode drug-resistant strains of hepatitis B virus. Antimicrob. Agents Chemother. 45, 1705–1713.

Dienstag, J.L., Schiff, E.R., Mitchell, M., Casey Jr., D.E., Gitlin, N., Lissoos, T., Gelb, L.D., Condreay, L., Crowther, L., Rubin, M., Brown, N., 1999. Extended lamivudine retreatment for chronic hepatitis B: maintenance of viral suppression after discontinuation of therapy. Hepatology 30, 1082–1087.

Fu, L., Cheng, Y.C., 1998. Role of additional mutations outside the YMDD motif of hepatitis B virus polymerase in L(-)SddC (3TC) resistance. Biochem. Pharmacol. 55, 1567–1572.

Hadziyannis, S.J., Tassopoulos, N.C., Heathcote, E.J., Chang, T.T., Kitis, G., Rizzetto, M., Marcellin, P., Lim, S.G., Goodman, Z., Wulfsohn, M.S., Xiohg, S., Fry, J., Brosgart, C.L., 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. New Engl. J. Med. 348, 800–807.

Hoofnagle, J.H., Di Bisceglie, A.M., 1997. The treatment of chronic viral hepatitis. New Engl. J. Med. 336, 347–356.

Hussain, M., Lok, A.S., 1999. Mutations in the hepatitis B virus polymerase gene associated with antiviral treatment for hepatitis B. J. Viral Hepatitis 6, 183–194.

Korba, B.E., Cote, P., Hornbuckle, W., Tennant, B.C., Gerin, J.L., 2000a. Treatment of chronic woodchuck hepatitis virus infection in the Eastern woodchuck (*Marmota monax*) with nucleoside analogues is predictive of therapy for chronic hepatitis B virus infection in humans. Hepatology 31, 1165–1175.

Korba, B.E., Cote, P.J., Hornbuckle, W.E., Schinazi, R.F., Gerin, J.L., Tennant, B.C., 2000b. Enhanced antiviral benefit of combination therapy with lamivudine and famciclovir against WHV replication in chronic WHV carrier woodchucks. Antivir. Res. 45, 19–32.

Korba, B.E., Cote, P., Hornbuckle, W., Schinazi, R., Gangemi, J.D., Tennant, B.C., Gerin, J.L., 2000c. Enhanced antiviral benefit of combination therapy with lamivudine and alpha interferon against WHV replication in chronic carrier woodchucks. Antivir. Ther. 5, 95–104.

Liaw, Y.F., Chien, R.N., Yeh, C.T., Tsai, S.L., Chu, C.M., 1999. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 30, 567–572.

Ling, R., Mutimer, D., Ahmed, M., Boxall, E.H., Elias, E., Dusheiko, G.M., Harrison, T.J., 1996. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 24, 711–713.

Lok, A.S., Heathcote, E.J., Hoofnagle, J.H., 2001. Management of hepatitis B: 2000—summary of a workshop. Gastroenterology 120, 1828–1853.

Marcellin, P., Chang, T.T., Lim, S.G., Tong, M.J., Sievert, W., Shiffman, M.L., Jeffers, L., Goodman, Z., Wulfsohn, M.S., Xiong, S., Fry, J., Brosgart, C.L., 2003. Adefovir dipivoxil for the treatment of hepatitis e antigen-positive chronic hepatitis B. New Engl. J. Med. 348, 808–816.

Mason, W.S., Cullen, J., Moraleda, G.l., Saputelli, J., Aldrich, C.E., Miller, D.S., Tennant, B.C., Frick, L., Averett, D., Condreay, L.D., Jilbert, A.R., 1998. Lamivudine therapy of WHV-infected woodchucks. Virology 245, 18–32.

Menne, S., Roneker, C.A., Korba, B.E., Gerin, J.L., Tennant, B.C., Cote, P.J., 2002. Immunization with surface antigen vaccine alone and after treatment with 1-(2-fluoro-5-methyl-β-L-arabinofuranosyl)-uracil (L-FMAU) breaks humoral and cell-mediated immune tolerance in chronic woodchuck hepatitis virus infection. J. Virol. 76, 5305–5314.

Oh, J.M., Kyun, J., Won, S., 2002. Long-term lamivudine therapy for chronic hepatitis B in patients with and without cirrhosis. Pharmacotherapy 22, 1226–1234.

Ono, S.K., Kato, N., Shiratori, Y., Kato, J., Goto, T., Schinazi, R.F., Carrilho, F.J., Omata, M., 2001. The polymerase L528M mutations cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J. Clin. Invest. 107, 449–455.

Ono-Nita, S.K., Kato, N., Shiratori, Y., Masaki, T., Lan, K.H., Carriho, F.J., Omata, M., 1999. YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: a study by *in vitro* full-length viral DNA transfection. Hepatology 29, 939–945.

Peek, S.F., Cote, P.J., Jacob, J.R., Toshkov, I.A., Hornbuckle, W.E., Baldwin, B.H., Wells, F.V., Chu, C.K., Gerin, J.L., Tennant, B.C., Korba, B.E., 2001. Antiviral activity of clevudine [L-FMAU (1-(2-fluoro-5-methyl-β,L-arabinofuranosyl) uracil)] against WHV replication and gene expression in chronically infected woodchucks. Hepatology 33, 254–266.

Perrillo, R., Hann, H.W., Mutimer, D., Wilems, B., Leung, N., Lee, W.M., Moorat, A., Gardner, S., Woessner, M., Bourne, E., Brosgart, C.L., Schiff, E., 2004. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 126, 81–90.

Perrillo, R., Schiff, E., Yoshida, E., Statler, A., Hirsch, K., Wright, T., Gutfreund, K., Lamy, P., Murray, A., 2000. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology 32, 129–134.

Peters, M.G., Hann, Hw.W., Martin, P., Heathcote, E.J., Buggisch, P., Rubin, R., Bourliere, M., Kowdley, K., Trepo, C., Gray, Df.D., Sullivan, M., Kleber, K., Ebrahimi, R., Xiong, S., Brosgrat, C.L., 2004. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 126, 91–101.

Seeger, C., Mason, W.S., 2000. Hepatitis B virus biology. Microbiol. Mol. Biol. Rev. 64, 51–69.

Tatti, K.M., Korba, B.E., Stang, H.L., Peek, S., Gerin, J.L., Tennant, B.C., Schinazi, R.F., 2002. Mutations in the conserved woodchuck hepatitis virus polymerase FLLA and YMDD regions conferring resistance to lamivudine. Antivir. Res. 55, 141–150.

Tennant, B.C., Gerin, J.L., 2001. The woodchuck model of hepatitis B virus infection. ILAR J. 42, 89–102.

Westland, C., Yang, H., Delaney IV, W., Gibbs, C., Miller, M., Wulfsohn, M., Fry, J., Brosgart, C., Xiong, S., 2003. Week 48 resistance surveillance in two phase 3 clinical studies of adefovir dipivoxil for chronic hepatitis B. Hepatology 38, 96–103.

Xiong, X., Flores, C., Yang, H., Toole, J.J., Gibbs, C.S., 1998. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. Hepatology 28, 1669–1673.

Xiong, S., Yang, H., Westland, C.E., Delaney IV, W.E., Colledge, D., Bartholomeusz, A., Thibault, V., Benhamou, Y., Angus, P., Wulfsohn, M., Gibbs, C.S., Fry, J., Brosgart, C.L., Locarnini, S., 2003. Resistance surveillance of HBeAg chronic hepatitis B (CHB) patients treated for two years with adefovir dipivoxil (ADV). J. Hepatology 38, 182 (Abstract).

Xiong, X., Yang, H., Westland, C.E., Zou, R., Gibbs, C.S., 2000. In vitro evaluation of hepatitis B virus polymerase mutations associated with famciclovir resistance. Hepatology 31, 219–224.

Yang, H., Westland, C.E., Delaney, W.E., Heathcote, E.J., Ho, V., Fry, J., Brosgart, C., Gibbs, C.S., Miller, M.D., Xiong, S., 2002. Resistance surveillance in chronic hepatitis B patients treated with adefovir dipivoxil for up to 60 weeks. Hepatology 36, 464–473.

Yeh, C.T., Chien, R.N., Chu, C.M., Liaw, Y.F., 2000. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. Hepatology 31, 1318–1326.

Zhou, T., Saputelli, J., Aldrich, C.E., Deslauriers, M., Condreay, L.D., Mason, W.S., 1999. Emergence of drug-resistant populations of woodchuck hepatitis virus in woodchucks treated with the antiviral nucleoside lamivudine. Antimicrob. Agents Chemother. 43, 1947–1954.