Pharmacodynamics of (-)-β-2',3'-Dideoxy-3'-Thiacytidine in Chronically Virus-Infected Woodchucks Compared to Its Pharmacodynamics in Humans

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The pharmacodynamics of (-)-β-2',3'-dideoxy-3'-thiacytidine (3TC) was studied in chronically woodchuck hepatitis virus-infected woodchucks and compared to that in previous studies in hepatitis B virus (HBV)-infected humans. Net depletion rates of serum virus DNA in woodchucks receiving 3TC were modeled as a sum of an exponentially declining virus input and a first-order elimination. Preceding shoulders and pseudo-first-order virus half-lives in serum ranged from 1 to 7 days and were dose dependent. Higher plasma 3TC concentrations were needed in woodchucks for virus depletion similar to that attained in humans. Human HBV depletion curves derived from multiple experiments with chronically WHV-infected woodchucks fit the pharmacokinetic model (2.4 and 2.0 days, respectively). On cessation of therapy, virus load rebounds in woodchucks were dose dependent and resembled posttherapy virus “flares” reported to occur in humans. The estimates of drug exposures that could lead to optimal antiviral effects presented indicate that 3TC should not be underdosed and compliance should be monitored. The study of chronically infected woodchucks may prove useful for optimizing drug regimens for hepadnavirus infections.

The woodchuck hepatitis virus (WHV) is a member of the hepadnavirus family, which includes duck hepatitis virus, ground squirrel hepatitis virus, and human hepatitis B virus (HBV) (12, 21, 27, 28, 32). WHV and duck hepatitis virus in their homologous animals are two of the most useful models for study of the efficacy of antiviral nucleoside agents under consideration for treating HBV infections (4, 10, 22, 32). These nucleosides, in their triphosphate form, target the viral DNA polymerase region (pol). The pol region in the WHV genome has the highly conserved catalytic YMDD motif and has about 54% amino acid sequence homology with HBV. Like HBV, WHV causes a chronic infection in its host that is associated with the development of hepatocellular carcinoma (23). There is a paucity of data concerning depletion of hepadnaviruses during treatment with (-)-β-2',3'-dideoxy-3'-thiacytidine (3TC, lamivudine). The aims of this study were to utilize data obtained from multiple experiments with chronically WHV-infected woodchucks to determine the in vivo pharmacodynamics of 3TC and to make comparisons with the results of previous studies in HBV-infected humans (7, 17).

(Parts of this work were presented at the Tenth International Conference on Antiviral Research, Atlanta, Ga., 6 to 11 April 1997 [8].)

MATERIALS AND METHODS

Infection and treatment of woodchucks. Chronic WHV infections were derived by experimental inoculation of neonatal animals as previously described (11, 33). All woodchucks used in these studies were chronically infected with WHV at the time of 3TC treatment. 3TC was administered in a liquid diet mixture by oral gavage. Data used in the pharmacodynamic analysis (see below) were obtained from three independent treatment studies in which woodchucks were treated with 3TC at either 1 mg/kg of body weight (twice daily; $n = 4$, all males), 5 mg/kg (once daily; $n = 20$, all males), or 15 mg/kg (once daily; $n = 6$, all males) over a 12-week period. Woodchucks treated at 5 mg/kg were also compared with those treated at that dose in two previous studies, one with females ($n = 4$) and one with males ($n = 6$).

High-performance liquid chromatography analysis of 3TC in plasma. Concentrations of 3TC in plasma were measured 1 h after dosing by a previously published isocratic high-performance liquid chromatography method with UV detection, to ensure consistency in plasma drug concentrations with those in a previous study (19).

Viral load determinations. Serum WHV DNA levels were measured by a dot blot hybridization technique (11, 33) over a 1- to 2-week period prior to the beginning of treatment and for up to 12 weeks during and following cessation of therapy. Hepatic WHV load (picograms per microgram of cell DNA) was also monitored with liver biopsies during these periods.

Basis for the development of the 3TC dose regimen for woodchucks. Woodchucks clear 3TC from the plasma more rapidly (terminal half-life $t_{1/2}$ = 2.8 h) than do humans (terminal $t_{1/2}$ = 7.2 h) (14, 19). At clinical doses, the cellular uptake of 3TC in cultured human cells and its subsequent phosphorylation to 3TC nucleotides have been shown to remain linear, with a $t_{1/2}$ of accumulation for the triphosphate of 5 to 4 h (2). Because the 3TC triphosphate (3TCTP) is markedly more resistant to phosphodiesterases than are the natural N-nucleosides (30), 3TC nucleotides egress from cells slowly, with a $t_{1/2}$ of accumulation of 2.8 h (2). The linear uptake and slow egress suggest that the net cellular accumulation of 3TCTP should be strongly related to average plasma drug concentrations. Since 3TC is administered daily over a period of weeks, and since the $t_{1/2}$ of 3TC in plasma is less than 8 h (7, 14, 17, 19), it can be assumed that equilibration between doses is reached relatively early during treatment (five 3TC $t_{1/2}$ is less than 2 days). Once equilibration is established, peak and minimum plasma drug concentrations between doses become reproducible, and average plasma drug concentrations ($C_{plas}$) can then be...
calculated by the following equation: 
\[ C_{\text{AUC}} = \text{AUC} \times \text{dose interval} \times 6 \]
where AUC is the total area under the plasma drug concentration-time curve resulting from one dose of 3TC. Previously published AUC values were substituted into this equation to estimate the average plasma drug concentrations as falling between 0.22 and 1.22 \( \mu \text{M} \) (14). Nowak et al. examined the dynamics of HBV in patients who received 100 to 600 mg of 3TC/day (17). Therefore, woodchucks were given between 2 and 15 mg of 3TC per kg per day orally, doses calculated to achieve average plasma drug concentrations known to have antiviral effect (in the range from 0.52 to 3.84 \( \mu \text{M} \)) based on previous in vitro and pharmacokinetic studies in 2.2.15 cells and woodchucks (5, 19).

**Derivations and models for antiviral pharmacodynamics in woodchucks.** An empirical model was used to describe virus depletion from serum for the various 3TC doses while avoiding overestimation of drug efficacy at lower doses. It was assumed that at an optimal therapeutic dose, sufficient 3TCTP is present to inhibit reverse transcription and virus replication ceases. The fraction of initial virus load (\( F \)) would decay over time (\( t \)) with a rate constant of \( K \), resulting in the equation 
\[ dF/dt = -K \times F \]  
At lower doses, virus load decay in the serum was assumed to be offset by virus input from infected cells where virus replication was not completely inhibited. The virus input rate, \( R \), is considered to decline with a rate constant of \( D \) during treatment, as active intracellular 3TCTP accumulates leading to a condition where 
\[ dF/dt = R^2 \times e^{-D \times F} / K - F \] 
This equation was integrated by the method of Laplace transforms, between the start (\( t = 0 \)) and the end of treatment, noting that \( F \) is not to be defined by this equation after treatment ceases (16):
\[ \mathcal{L}(F)(s + K) = R^2(s + D) - K \times \mathcal{L}(F)(s) + F_0 \]
\[ \mathcal{L}(F)(s) = R^2(s + D) + s(s + K) + F_0(s + K) \]
Here, \( \mathcal{L} \) is the Laplace operator. Taking inverse Laplace transforms (\( \mathcal{L}^{-1} \)) and noting that \( F_0 \) is equal to 1 since the initial virus fraction at the start of therapy is equal to 1, we obtain
\[ \mathcal{L}^{-1}[\mathcal{L}(F)] = F = R^2(D - K) \times (e^{-D} - e^{-D \cdot F}) + e^{-K \cdot F} \]  
Equation 1 was fitted to averaged data from each group of woodchucks by a nonlinear least-squares regression procedure based upon the method of false position (20, 24).

The effect of dose on the terminal elimination rate of virus from plasma (\( K' = 0.693/\text{terminal} \)) was modeled by using an inhibitory sigmoid \( E_{\text{max}} \) model (5). In this model
\[ K' = -K_{\text{max}} \cdot \text{AUC}/(\text{AUC}_{50} + \text{AUC}) \]  
In equation 2, \( K_{\text{max}} \) is the maximum rate of virus depletion from the plasma, when virus replication is inhibited, and was approximated as 0.35 day\(^{-1}\), the depletion rate observed at the highest dose. AUC is the estimated daily area under the plasma concentration-time curves for 3TC (expressed in micrograms • hours per milliliter), based on a previously published pharmacokinetic study in woodchucks (19). \( \text{AUC}_{50} \) is the fitted value of AUC corresponding to a value of \( K' \) half that of \( K_{\text{max}} \) and \( \gamma \) is the exponent to which the AUC is raised. Equation 2 was fitted to the averaged data for each group of woodchucks using nonlinear least-squares regression.

**Pharmacodynamics in humans.** Average serum virus loads obtained by Nowak et al. (17) (see Fig. 3) were fit to the biphasic function
\[ V = A \cdot e^{-r_1 t} + B \cdot e^{-r_2 t} \]  
by nonlinear least-squares regression, resulting in an \( r^2 \) value of 0.95 (21, 24). In equation 3, \( V \) is virus load, \( A \) and \( B \) are coefficients, and \( r_1 \) and \( r_2 \) are rate constants (\( \alpha > \beta \)). The percentages of the total area under the virus depletion curve (virus depletion AUC) contributed by the respective exponentials, \( F_A \) and \( F_B \), were calculated as
\[ F_A = (A/\alpha)/(A/\alpha + B/\beta) \times 100 \text{ and } F_B = (B/\beta)/(A/\alpha + B/\beta) \times 100 \]  
and the respective \( t_{1/2} \) were calculated as 0.693/\( \alpha \) and 0.693/\( \beta \), respectively.

## RESULTS

**Woodchuck studies.** Prior to treatment of woodchucks with 3TC at an average age of 14 ± 7 months (mean ± standard deviation), the mean virus load in serum was 3.79 × 10\(^3\) ± 2.43 × 10\(^3\) per ml (\( F = 1 \)). The fraction of this initial serum virus load (\( F \)) for the chronically infected woodchucks treated with the various 3TC doses and the corresponding fitted curves are presented in Fig. 1. A maximal first-order decline was noted for the 15-mg/kg/day dose, with a \( t_{1/2} \) of 2 days (\( r^2 > 0.99 \); three points). One milligram of 3TC per kilogram twice per day resulted in an initial shoulder prior to an apparent first-order decline at about week 6, with a \( t_{1/2} \) of 14 days, based upon serum virus loads from week 6 to 12 (\( r^2 = 0.94 \); four points). When this \( t_{1/2} \) was used to estimate \( F \) at 12 weeks, without considering the preceding shoulder, the viral load was underestimated by 53%. To compensate for a shoulder, all nine data points (0 to 12 weeks), were fitted to equation 1. \( K \) was estimated as the first-order rate constant from the 15-mg/kg/day dose (2.3/week), and nonlinear regression was used to estimate the rate constant, \( D \), and the apparent initial virus input rate, \( R \). This equation estimated \( F \) at week 12 to within 15\% (\( r^2 = 0.98 \), observed versus model predicted). At 5 mg of 3TC/kg/day, a less-than-maximal pseudo-first-order decline in serum virus load was observed with an apparent \( t_{1/2} \) of 7 days (\( r^2 > 0.99 \); six points). These data also fit equation 1, with an \( r^2 \) value of >0.99. The \( D \) half-life (\( D_{1/2} \)) values and fitted...

**FIG. 1.** Decay of WHV load in woodchuck serum during treatment with 3TC at 1 mg/kg twice daily (●), 5 mg/kg/day (○), and 15 mg/kg/day (□). Mean values for fraction of initial virus load (\( F \)) were fitted to equation 1. Dotted lines represent model fits. The model parameters are summarized in Table 1. SEM, standard error of the mean.
initial virus input rates, $R_0$, decreased monotonically with dose, consistent with a more rapid virus inhibition and reduced shoulders at higher doses. Table 1 contains fitted parameters for the 3TC doses tested in the chronically infected woodchucks.

There was a difference in virus decline between the study with 20 male woodchucks given 5 mg of 3TC/kg/day and two previous studies conducted in 4 female and 6 male woodchucks given the same dose. The female cohort showed a $t_{1/2}$ of 2.2 weeks, similar to that observed in males given the 1-mg/kg twice-daily dose. The small male group ($n = 6$) showed a shoulder that lasted about 2 weeks before the onset of a pseudoexponential decline with a $t_{1/2}$ of 2.0 days, similar to the terminal $t_{1/2}$ observed for the cohort given 3TC at the 15-mg/kg dose. WHV DNA in the livers of woodchucks was depleted to a significant extent ($P < 0.05$) only after a 12-week treatment with 2 mg of 3TC/kg/day, even though virus loads in the plasma had declined much earlier (data not shown). Postshoulder virus depletion rates ($K' = 0.693/terminal t_{1/2}$ [days]) plotted against the estimated AUC fit equation 2 ($r = 0.99$), resulting in the following relationship: $K' (\text{day}^{-1}) = -0.35 \cdot \text{AUC}^{5.6} / (8.9^{3.0} + \text{AUC}^{5.0})$.

Mean serum WHV loads rebounded after termination of 3TC treatment in a dose-dependent manner, with the highest “flare” following the 15-mg/kg/day 3TC dose and the lowest following the 2 mg/kg/day dose (Fig. 2). All serum virus loads subsequently declined to pretreatment values by week 8.

**Human studies.** Virus depletion profiles in humans (Fig. 3) fit the equations expressing a biexponential function (eq. 3 and 4), with an $r^2$ value of 0.95 (20, 24). Although the regression was limited by the inclusion of only six data points per patient, individuals had similar virus decay profiles. The percentages of the total area under the virus depletion curve (virus depletion AUC) contributed by the exponentials $F_\alpha$ and $F_\beta$ were 78 and 22%, respectively, and the $t_{1/2}$ were 1.25 and 7.0 days, respectively. The weighted average $t_{1/2}$, normalized to a fraction of the virus AUC, was similar to the $t_{1/2}$ observed in woodchucks given the highest 3TC dose (2.4 and 2.0 days, respectively).

**DISCUSSION**

An empirical model was developed for 3TC pharmacodynamics based on the assumption that virus depletion in the plasma results from inhibition of viral reverse transcription by 3TCTP (1, 29). Reverse transcription is needed to produce viral DNA, the infectious form of the genome, from the viral RNA pregenome (27, 34). Virus replication and hence serum virus loads and infection rates should decrease as 3TC accumulates in productively infected cells. The time taken for a sufficient amount of 3TCTP to accumulate and inhibit reverse transcription is expected to depend upon drug efficacy and dose and the accumulation and egress of 3TCTP. In addition, the elimination of virus from host tissue depends upon the turnover of infected cells and, possibly, immune system-mediated processes (4, 12). The maximal rate of virus depletion ($K$)
plotted against the estimated AUC was modeled with an inhibitory sigmoidal $E_{\text{max}}$ model, resulting in an AUC$_{\text{eq}}$ value per day of 8.9 $\mu$g · h/ml. A value for $\gamma$ of 3.6 indicates a relatively strong dose-response relationship between daily AUC and $K$, indicating that 3TC should not be underdosed and compliance should be monitored. The apparent difference in 3TC pharmacodynamics between male and female woodchucks is not presently understood, since the influence of gender on 3TC pharmacokinetics has not been studied in woodchucks.

Rebounds in serum virus load can be explained by assuming virus production to be related to the availability of susceptible uninfected cells. This approach was recently used to explain virus rebounds following cessation of therapy for human immunodeficiency virus (18). Under these assumptions, a more effective treatment should result in fewer infections of new cells, with a net decline in infected tissue as these cells are cleared. Once 3TC is discontinued, viral replication could resume in remaining infected cells, leading to a renewed virus input into the serum. The rate of infection of susceptible cells may be high initially, giving rise to maximal synthesis rates and maximal rates of virion infusion into the serum, producing the flare. However, the ratio of uninfected and/or susceptible cells to infected cells would decline as the infection spreads, resulting in a decreased viral synthesis rate. Apparent equilibrium could occur when cell infection rates approach the turnover rate of infected cells. When suboptimal doses are terminated, many cells may still be infected, leading to a less pronounced posttreatment flare in serum virus load. This was observed in woodchucks given 2 mg of 3TC/kg/day. Virus RNA is transcribed by covalently closed circular DNA (cccDNA), which is localized in the nuclei of host cells (15). The copy number of cccDNA is regulated by viral envelope proteins and maintained at between 5 and 30 per productively infected cell (9, 31). 3TC does not alter the stability of cccDNA in infected nuclei. However, if reverse transcription is inhibited, cccDNA production should cease, leading to a reduction in cccDNA content of infected nuclei as cells divide (13). A more rapid increase in serum virus load immediately following higher doses of 3TC (Fig. 2) suggests an increase in the fraction of susceptible uninfected cells during the 12-week 3TC treatment.

Earlier studies with woodchucks indicate that following acute infection, an almost complete replacement of hepatocytes may occur within 4 weeks (4, 9). WHV is known to replicate in hepatocytes and in cells of lymphoid origin (11). Consequently, it would be difficult to determine the exact contribution of each cell type to the serum virus load (22).

Nowak et al. (17) studied the dynamics of HBV in six chronically infected humans who received 3TC at doses of $\geq$100 mg per day (Fig. 3). No initial shoulders were evident prior to the onset of maximal viral depletion rates. Virus loads obtained for the first 2 days were used to calculate a $t_{1/2}$ of 0.92 ± 0.59 days (mean ± standard deviation). This value was used as an estimate of the virus turnover rate. However, depletion rates progressively declined during the treatment for five of the six patients, resulting in a $t_{1/2}$ between days 2 and 28 of 4.7 ± 0.39 days. To model viral depletion during 3TC treatment, Nowak et al. (17) used the equation $v(t) = v_0(1 - e^{-\lambda t})$, where $v_0$ and $v(t)$ are virus loads at the start of 3TC treatment ($t = 0$) and at time $t$, respectively, $\lambda$ is the initial maximal rate of virus decline in plasma, and $p$ is the efficiency factor, which accounts for a drug efficacy of less than 100%. Values for $\lambda$ were 0.145, 0.17, 0.17, and 0.15 for 3TC at doses of 20, 100, 300, and 600 mg per day, respectively. A 5-mg/day 3TC cohort was included in that study, but no value for $p$ was reported for this dose. This equation predicts a maximal virus depletion rate in serum at the start of 3TC treatment, which decreases asymptotically as the virus load approaches $v_0(1 - p)$, without compensating for an initial shoulder. This equation was not used for the woodchuck data, since it does not compensate for the shoulder at the lowest dose. Furthermore, serum virus loads at the two higher doses both fell below the detection limit, suggesting that the values for $v_0(1 - p)$ both approached 0, despite a 3.5-fold difference in $t_{1/2}$ (Table 1).

Virus depletion profiles in humans were fitted to a biexponential function (eq. 3 and 4), using nonlinear least-squares regression, to allow a more direct comparison with those in woodchucks (20, 24). The weighted average $t_{1/2}$ in humans, normalized to fraction of virus depletion AUC, was similar to the $t_{1/2}$ observed in woodchucks given the highest 3TC dose (2.4 and 2.0 days, respectively). Thus, although plasma drug concentrations in woodchucks given the lowest dose of 3TC (2
mg/kg/day) were calculated to produce higher values than those in humans receiving 100 mg per day, virus decline rates in woodchucks were similar to those in humans only at a much higher dose (15 mg/kg/day). Multiphasic or asymptotic declines in serum virus levels in humans could result from many factors, including variations in drug exposures between tissues that harbor the virus, the emergence of resistant virus strains, or interactions between tissues and virus.

If 3TC treatment is terminated prior to the clearance of productively infected cells, active disease may recur. Plasma virus loads of the patients studied by Nowak et al. did not rebound above pretreatment values on discontinuation of therapy (17). However, recent reports of HBV flares have emerged from studies in Japan, where one patient died and six others were hospitalized with suspected acute liver failure following cessation of 3TC (11a). Furthermore, posttreatment HBV flares have been reported by Honkoop et al. for a 29-year-old patient following 6 months of 3TC therapy, during which serum HBV levels fell below detection limits (7). However, 4 weeks after cessation of treatment there were symptoms of hepatic collapse with no evidence of cirrhosis. Furthermore, serum HBV levels rose fourfold higher than pretreatment values, a magnitude of increase similar to that noted in woodchucks following cessation of 3TC at the highest dose (Fig. 2). There was a 16% incidence of elevations in alanine aminotransferase levels (more than three times the baseline level) among a cohort of 83 patients enrolled in their study. Of these, only three patients showed associated hyperbilirubinemia or jaundice. With the exception of the patient described above, in all patients the symptoms and signs resolved spontaneously. Healthy carriers of HBV may require treatment for at least 1 year and possibly for the remainder of their lives (13). Therefore, virus rebounds could become more frequent as more patients are treated for extended periods, and in a percentage of the affected patients they may prove life threatening. Since patient compliance may be inversely related to treatment duration, suboptimal dosing could become a problem. Thus, it is important to develop new treatment modalities, including combination chemotherapy, to achieve rapid and sustained antiviral effects (25). The woodchuck model could prove useful in the development of strategies for modulating and preventing virus rebounds.

In summary, we studied the pharmacodynamics of 3TC in chronically virus-infected woodchucks at various physiological doses, some of which proved suboptimal. An empirical model was developed to describe virus depletion at these doses. In woodchucks higher concentrations of 3TC in plasma were needed to produce virus depletion profiles similar to those found in humans. However, these profiles were similar at higher doses. This may be related to the generally lower rate of 3TC phosphorylation in woodchuck liver than in human liver (26). Increasing our understanding of the relationships among antiviral drug dose, antiviral pharmacodynamics, and treatment outcome in woodchucks and humans should lead to optimized therapeutic approaches for hepatadnavirus infections.

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ERRATUM

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Volume 42, no. 11, p. 2804–2809, 1998. Page 2806, Table 1: boxhead “R° (per day)” should read “Fraction of initial serum virus per day” and in line 5 of footnote b “(K)” should read “(K [same as $K_{\text{max}}$ in equation 2])” and “2.3 per day” should read “0.35 per day.”