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Pharmacokinetics of Telbivudine following Oral Administration of Escalating Single and Multiple Doses in Patients with Chronic Hepatitis B Virus Infection: Pharmacodynamic Implications

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The pharmacokinetics of telbivudine were evaluated in adult patients with chronic hepatitis B virus (HBV) infection following once-daily oral administration at escalating doses of 25, 50, 100, 200, 400, and 800 mg/day for 4 weeks. Telbivudine was rapidly absorbed after oral administration, with the median times T_{max} to the maximum plasma concentration (C_{max}) ranging from 0.8 to 3.0 h postdosing across cohorts. Single-dose and steady-state maximum C_{max}s and the areas under the plasma concentration-time curve from time zero to time t (AUC_{0-t}s) increased proportionally with dose. At steady-state, the values of C_{max} and AUC_{0-t} were higher than those obtained after the administration of a single dose, indicative of a slight accumulation, with the ratios of the steady-state value to the value after the administration of a single dose ranging from 1.14 to 1.49 for C_{max} and from 1.40 to 1.70 for AUC_{0-t}. While the elimination of telbivudine from plasma was apparently monophasic over the 8-h sampling period, the substantial steady-state trough plasma levels observed in the groups receiving doses of 100 to 800 mg were clearly indicative of the presence of a second slower elimination phase, with the mean estimated half-lives ranging from 29.5 to 41.3 h by compartmental modeling analysis. Pharmacokinetic and pharmacodynamic analyses by using maximum-effect modeling established a quantitative relationship between a reduction in serum HBV DNA levels and parameters of drug exposure, in particular, the steady-state C_{max} and AUC_{0-t}. In summary, this study showed that telbivudine exhibits dose-proportional plasma pharmacokinetics with sustained steady-state drug exposure and exposure-related antiviral activity, supporting the need for further clinical studies by use of a once-daily regimen in patients with chronic HBV infection.

Telbivudine (β-L-2′-deoxythymidine) is an L-configured nucleoside with potent and specific activities against hepatitis B virus (HBV) and other hepadnaviruses and no appreciable activity against human immunodeficiency virus or other viruses (3). The in vitro median effective concentration of telbivudine for reducing extracellular DNA levels in HBV-expressing hepatoma cell line 2.2.15 was 0.19 μM (~0.05 μg/ml). In woodchucks chronically infected with woodchuck HBV, up to 28 days of telbivudine treatment produced consistent, multilog reductions in circulating serum woodchuck HBV DNA levels (3).

In vitro toxicological assessments produced no adverse findings (1–3). The 50% cytotoxic concentration of telbivudine in 2.2.15 cells was >2,00 μM (~500 μg/ml), indicating that it has an excellent therapeutic index in cell culture (3). Other in vitro results suggested that telbivudine is unlikely to be associated with hematologic or mitochondrial toxicities, peripheral neuropathy, or myopathy (3). Mutagenic test results were negative (1).

Acute and subchronic toxicology studies did not identify any preclinical safety issues for telbivudine. In acute and subchronic (28-day) toxicity studies conducted with rats and monkeys with daily doses up to 2,000 mg/kg of body weight, no treatment-related clinical abnormalities were observed (1, 2).

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Preclinical pharmacological studies conducted with cynomolgus monkeys following the administration of oral and intravenous doses showed that telbivudine was well absorbed, with an oral bioavailability of 68% (4). Renal clearance appeared to be the major pathway of telbivudine elimination (4). Preliminary results from a mass balance study with rats and ^{14}C-labeled telbivudine indicated no major plasma or urine metabolite (unpublished data).

The encouraging preclinical anti-HBV activity and favorable safety profile of telbivudine prompted a phase I/II study with patients with chronic HBV infection. The objectives were to evaluate the safety, pharmacokinetics, and antiviral activity of telbivudine administered orally at escalating doses starting at 25 mg/day for 4 weeks. Clinical efficacy and safety results have recently been reported in more detail elsewhere (6). This report provides a detailed pharmacokinetic analysis of telbivudine and elaborates the pharmacodynamic relationships with implications for telbivudine dose selection in subsequent phase IIb (7) and phase III clinical safety and efficacy trials.

MATERIALS AND METHODS

Study population. Patients meeting the following inclusion criteria were eligible for the study: age of 18 years or older; documented chronic HBV infection, as determined by the presence of serum HBsAg for ≥6 months; serum HBeAg positivity for ≥1 month; a serum HBV DNA level of $\geq 10^7$ copies/ml; and a serum alanine aminotransferase level less than five times the upper limit of normal. Written informed consent was obtained from all patients. Major exclusion criteria included pregnancy or breast-feeding; coinfection with hepatitis C

virus, hepatitis D virus, or human immunodeficiency virus; any previous treatment with a nucleoside analog or any other drug treatment for HBV in the year before the baseline; treatment with immunomodulatory agents or corticosteroids within 6 months before the baseline; decompensated liver disease; hepatocellular carcinoma; a history of other clinically important diseases and current abuse of alcohol or illicit drugs. The trial was conducted at the Queen Mary Hospital in Hong Kong and the National University Hospital in Singapore. The trial was approved by the ethics committees of both centers. The first patient was recruited on 20 July 2000, and last patient completed the trial on 5 July 2002.

Study design. This was a phase I/II, double-blind, placebo-controlled, dose-escalation trial for evaluation of the safety, pharmacokinetics, and anti-HBV activity of telbivudine administered once daily for 4 weeks at escalating doses of 25, 50, 100, 200, 400, and 800 mg, with a 12-week follow-up period. Seven patients who were randomly assigned to receive telbivudine or matching placebo at a 6:1 ratio were enrolled in each dosing cohort. Telbivudine 25-mg tablets were used in the 25- to 200-mg cohorts; 100-mg tablets were given to the 400-mg cohorts; and 200-mg tablets were administered to the 800-mg cohort. All telbivudine tablets were formulated for immediate release. The study drug was taken orally once daily in the morning. There was no restriction regarding food intake.

Fourteen to 7 days prior to the baseline (the time of administration of the first dose), patients underwent a screening evaluation that included a medical history, a physical examination, and clinical laboratory measurements (HBV DNA level, serum chemistry, hematology, and urinalysis). Clinical laboratory measurements were repeated at the baseline and were monitored weekly during the study.

Blood sample collection. An intensive pharmacokinetic evaluation was conducted over a period of 8 h after the first dose and on any day between weeks 2 and 4 (steady state). The selection of the blood sampling duration was largely dictated by the outpatient nature of the study and consideration of the convenience of the participants. Blood samples (5 ml at each time point) were drawn into heparin-containing Vacutainer tubes immediately prior to and after dosing at 0.5, 1, 2, 3, 4, 6, and 8 h. Plasma was obtained by centrifugation and was stored at −20°C until analysis. Serum samples for viral load measurement were obtained at the baseline, weekly through week 8, and thereafter every other week through week 16.

Blood sample analyses. For plasma samples from the 25- to 400-mg dose cohorts, plasma concentrations of telbivudine were assessed by a validated high-performance liquid chromatographic (HPLC) method with UV detection. Briefly, 50 μl of internal standard (β-L-2′-deoxyadenosine [LdA] at 40 μg/ml) and 6 μl of thymidine phosphorylase (EC 2.4.2.4; >1 unit/μl; Sigma Chemical Co., St. Louis, Mo.) were added to 200 μl of calibration standards (0.1 to 100 μg/ml), quality controls (QC; 0.1 to 80 μg/ml), and unknown plasma samples. The mixture was vortexed thoroughly and incubated at 37°C for 1 h to digest any endogenous thymidine that may interfere with the telbivudine assay. After incubation, acetonitrile (1.5 ml) was added to precipitate protein. The samples were centrifuged, and the supernatant was recovered and evaporated to dryness. Prior to chromatographic analysis, dry residues were reconstituted with 150 μl of mobile phase (20 mM phosphate buffer containing 2% [vol/vol] acetonitrile) and centrifuged. The resulting supernatant was transferred into HPLC injection vials. Reverse-phase chromatography was performed with 50-μl aliquots on a Novapak C_{18} column (3.9 by 150 mm; 4-μm pore size; Waters, Milford, Mass.). Elution was carried out isocratically at 1 ml/min. Under these conditions, the retention times were approximately 4.9 and 7.7 min for telbivudine and LdA, respectively. Telbivudine and LdA were monitored at 267 nm. This assay was characterized by a lower limit of quantitation of 0.1 μg/ml, intra- and interday precisions (coefficients of variation [CVs]) from 1.1 to 10.6%, and accuracies (percent deviation) from −3.3 to 6.1%, based on the results for the QC samples.

Plasma samples from the 800-mg cohort were analyzed by a validated HPLC method with mass-spectrometry (MS)-MS detection. In this assay, the sample preparation procedure was similar to that for the HPLC with UV detection method described above. However, because of the high sensitivity of MS-MS detection, a much smaller sample volume (100 μl) was used. Reverse-phase chromatography was performed on a TSK-GEL Amide-80 column (4.6 by 150 mm; 5 μm pore size; Tosoh Bioscience, Montgomeryville, Pa.). Elution was carried out isocratically at 1 ml/min with a mobile phase of 90:10 (vol/vol) methanol–25 mM ammonium formate (pH 3.5). Under these conditions, the retention times were approximately 1.68 and 1.73 min for telbivudine and LdA, respectively. Telbivudine and LdA were monitored by using a PE Sciex API 3000 MS-MS mass analyzer at mass transitions of 243.0 to 127.1 *m/z* and 252.0 to 136.0 *m/z*, respectively. The mass analyzer was operated under the positive mode by using atmospheric pressure chemical ionization. This assay has a lower limit of quantitation of 0.01 μg/ml, with a calibration curve range from 0.01 to 5 μg/ml. The intra- and interday precisions (CVs) and accuracies (percent deviation) were from 2.3 to 5.6% and −4.2 to 1.4%, respectively, based on the results for QC samples with concentrations ranging from 0.03 to 4 μg/ml. Both bioanalytical methods were validated to their respective specifications without further cross-validation.

Serum HBV DNA was quantitated by using the COBAS AMPLICOR PCR assay (Roche Diagnostics, Branchburg, N.J.), which has a quantitation limit of 400 genome copies/ml).

Pharmacokinetic and pharmacodynamic analyses. The plasma concentration-time data for telbivudine obtained after the first dose and at steady state were analyzed by model-independent and compartmental modeling approaches by using Kinetica (version 4.3; Thermo Electron Corporation, Waltham, Mass.). The maximum plasma drug concentration (C_{max}) and the time to C_{max} (T_{max}) were obtained directly from the plasma concentration-time profiles. The area under the plasma concentration-time curve from time zero to time t (AUC_{0-t}), where t is the time that the concentration was last measurable in a sample, was calculated according to the linear trapezoidal rule. The half-life ($t_{1/2}$) observed over the 8-h sampling period was calculated as 0.693/λ, where λ is the slope of the linear portion of the natural log-transformed post-peak plasma drug concentration-time curve estimated by linear regression. Potential drug accumulation was assessed by determination of the ratio of the steady-state value to the first-dose value (accumulation factor) of C_{max} and AUC_{0-t}.

To characterize the true terminal elimination phase, a compartmental modeling analysis was applied to individual steady-state plasma concentration-versus-time data for patients with measurable predose trough levels (100- to 800-mg doses). Because of the lack of samples during the terminal phase, steady-state predose plasma levels were "flipped over" and considered 24-h datum points. A two-compartment open model with first-order input and first-order elimination, parameterized into microconstants (absorption rate constant [K_a] and intercompartment rate constants [k_{12} and k_{21}]) and the volume of the central compartment (V_1), was fitted to the data. The estimated microconstants were then used to calculate the elimination rate constant (β) and the terminal-phase half-life ($t_{1/2\beta}$; which is equal to 0.693/β).

The principal parameters underlying plasma drug exposure, including C_{max} and AUC_{0-t}, were assessed for dose proportionality after the first dose and at steady state in the 25- to 800-mg daily dose range by using the following power model: $Y_{ij} = a \times D_j^b \times e_{ij}$, where D_j is the dose at level j; Y_{ij} is the pharmacokinetic parameter for subject i at dose level j; a and b are the mean intercept and slope, respectively; and e_{ij} is the residual error for subject i at dose level j. For practical reasons, the model presented above is $\log_{10}$ linearized as follows: $\log(Y_{ij}) = a + b \times \log(D_j) + e_{ij}$, and fitted by using the GLM (general linear models) procedure in SAS software (version 8.0; SAS Institute Inc., Cary, N.C.). A dose-proportional relationship is concluded if the 95% confidence interval of the estimated mean slope (b) includes unity.

The relationship between telbivudine antiviral activity (pharmacodynamics) and plasma exposure was explored by using the following E_{max} model: $E = E_{max} \times PK/(EPK_{50} + PK)$, where E and E_{max} are the observed and the maximum antiviral effects, respectively, measured as the viral load reduction (the HBV DNA level on a $\log_{10}$ scale) at the end of treatment (week 4); PK represents the pharmacokinetic parameters of exposure, i.e., single-dose and steady-state C_{max} and AUC_{0-t}; and EPK_{50} is the value of the pharmacokinetic parameter that produces 50% of E_{max}. Nonlinear regression was performed by using the NLIN procedure in SAS software.

RESULTS

Patient characteristics and disposition. All patients enrolled were Asian and were primarily of Chinese ethnicity. The treatment groups were comparable with respect to demographics and baseline serum HBV DNA levels. Table 1 summarizes the patients' demographics and baseline HBV-related data. Forty-two of the 43 patients (36 patients receiving active treatment and 7 patients receiving placebo) completed the 4-week treatment and the 12-week follow-up periods. One subject randomized to the placebo in the 200-mg cohort withdrew from the study for personal convenience reasons unrelated to the study treatment. One patient in the 400-mg telbivudine cohort completed the study but was considered noncompliant by the investigator.

Plasma pharmacokinetics. The plasma pharmacokinetics of telbivudine were investigated over an 8-h period on day 1 and on any day between weeks 2 and 4 inclusive. Pharmacokinetic

TABLE 1. Patient characteristics at baseline[a]

Characteristic	Placebo	Value at the following telbivudine dose (mg):						
		25	50	100	200	400	800	Total
No. of subjects	7	6	6	6	6	6	6	36
Age (yr)[b]	30.9 ± 9.8	31.7 ± 7.5	32.0 ± 8.4	38.7 ± 11.6	29.4 ± 7.8	32.5 ± 10.0	41.8 ± 16.5	34.3 ± 10.9
Gender (% male)	86	83	50	83	67	67	100	75
Wt (kg)[b]	68.0 ± 13.4	72.5 ± 17.2	63.3 ± 17.2	59.4 ± 17.2	62.6 ± 9.1	63.6 ± 8.5	66.3 ± 9.9	64.6 ± 13.4
Serum HBV DNA[b] ($\log_{10}$ no. of copies/ml)	8.3 ± 1.2	9.9 ± 1.1	8.8 ± 0.8	9.0 ± 1.1	7.9 ± 0.8	8.2 ± (0.6)	8.7 ± 1.3	8.8 ± 1.1
Serum ALT[c] (IU/ml)[d]	33 (27–78)	68 (22–75)	30 (24–139)	63 (21–163)	81 (20–169)	126 (22–545)	52 (31–243)	58 (20–545)

[a] All patients were Asian and seropositive for HBsAg and HBeAg.
[b] Values are presented as means ± SDs.
[c] ALT, alanine aminotransferase.
[d] Values are presented as medians (ranges).

data from 35 telbivudine-treated patients were evaluated (Table 2); the data for the pharmacokinetic parameters for the noncompliant subject in the 400-mg cohort were estimated (Table 2 footnotes) but are not included in the cohort summary statistics. Figure 1 shows the mean plasma telbivudine concentration-time profiles obtained after single (day 1) and repeated (steady-state) daily doses of 25 to 800 mg in HBV-infected patients. Following oral dosing, telbivudine was rapidly absorbed, with peak plasma levels reached between 0.8 and 3.0 h (median values) across all dose cohorts on day 1 and at steady state. Over the dose range of 25 to 800 mg/day studied, in which the twofold dose increments imply an anticipated doubling factor of 2.0 between adjacent doses with respect to exposure, the mean C_{max} increased from 0.20 to 3.97 μg/ml (mean doubling factor, 1.9 ± 0.4) on day 1 and from 0.22 to 5.46 μg/ml (doubling factor, 1.9 ± 0.4) at steady state, and the mean AUC_{0-t} increased from 0.51 to 20.94 μg · h/ml (doubling factor, 2.1 ± 0.5) on day 1 and from 0.75 to 29.73 μg · h/ml (doubling factor, 2.1 ± 0.4) at steady state. The pharmacokinetic dose proportionality of telbivudine was ascertained by statistical analyses of log-transformed parameters and dose. The model estimates of the slope were close to unity in all cases: 0.91 (95% confidence interval, 0.81 to 1.01) and 0.93 (95% confidence interval, 0.84 to 1.02) for day 1 and steady-state C_{max}, respectively, and 1.08 (95% confidence interval, 0.96 to 1.20) and 1.03 (95% confidence interval, 0.93 to 1.14) for day 1 and steady-state AUC_{0-t}, respectively. The telbivudine C_{max} and AUC_{0-t} were greater at steady state than after a single dose. The mean ratios of the individual paired values at steady state to the values after administration of a single dose (accumulation factor) ranged from 1.14 to 1.49 for C_{max} and from 1.40 to 1.70 for AUC_{0-t}. The detailed pharmacokinetic parameter data for each dose cohort are presented in Table 2.

After peak plasma levels were achieved, the disposition of telbivudine was essentially monophasic over the 8-h sampling

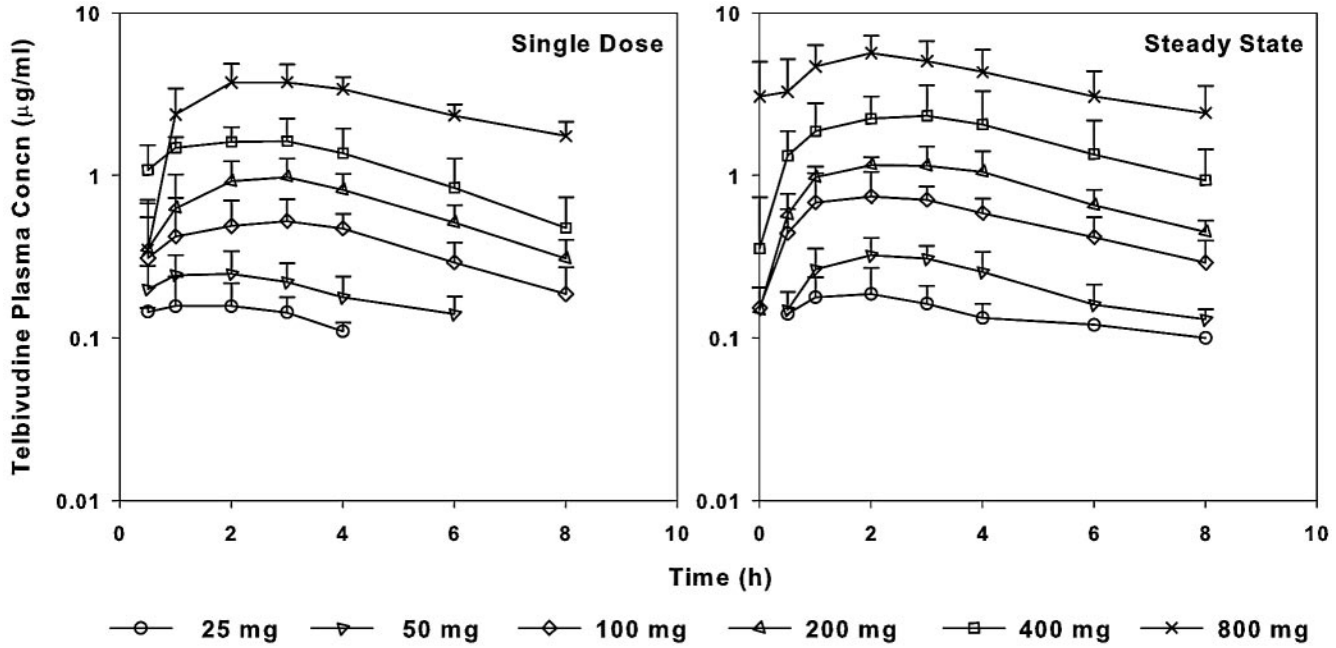

FIG. 1. Mean (SD) single-dose (day 1) and steady-state (week 2 to 4) plasma concentration-time profiles of telbivudine in HBV-infected patients following oral administration of escalating doses at 25, 50, 100, 200, 400, and 800 mg.

TABLE 2. Values of pharmacokinetic parameters for telbivudine on day 1 (first dose) and at steady state (weeks 2 to 4) following once-daily oral administration of escalating doses from 25 to 800 mg in HBV-infected patients[j]

Dose[a] (mg)	C_{max} (μg/ml)			C_{trough}[d] (μg/ml), SS	AUC_{0-t} (μg · h/ml)			T_{max}[e] (h)		$t_{1/2}$[f] (h)		$t_{1/2\beta}$[g] (h)
	Day 1	SS[h]	Ratio[c]		Day 1	SS	Ratio[c]	Day 1	SS	Day 1	SS	
25	0.20 (0.08)	0.22 (0.08)	1.14 (NA[i])	NA	0.56 (0.33)	0.75 (0.31)	1.48 (NA)	0.8 (0.5–1.1)	1.0 (1.0–1.9)	3.5 (0.9)	5.0 (2.2)	NA
50	0.27 (0.07)	0.35 (0.08)	1.31 (0.14)	NA	0.98 (0.38)	1.60 (0.49)	1.70 (0.26)	1.5 (1.0–3.0)	1.9 (1.0–3.0)	3.1 (1.3)	3.0 (0.4)	NA
100	0.61 (0.21)	0.82 (0.24)	1.38 (0.27)	0.15 (0.05)	2.91 (0.88)	4.14 (1.05)	1.45 (0.17)	3.0 (0.4–4.0)	2.1 (1.2–4.0)	3.0 (0.8)	3.6 (0.6)	41.3 (8.4)
200	0.99 (0.31)	1.24 (0.30)	1.32 (0.36)	0.15 (0.06)	5.02 (1.47)	6.67 (1.47)	1.40 (0.40)	2.9 (1.9–3.0)	1.9 (1.9–2.9)	3.1 (1.4)	3.5 (0.9)	29.5 (9.1)
400[b]	1.80 (0.44)	2.73 (0.96)	1.49 (0.31)	0.35 (0.38)	9.05 (3.02)	13.42 (6.22)	1.44 (0.33)	3.0 (0.4–3.0)	1.9 (1.9–3.0)	2.5 (0.5)	3.9 (1.0)	31.5 (14.8)
800	3.97 (1.01)	5.46 (1.64)	1.41 (0.42)	2.65 (2.01)	20.94 (3.47)	29.73 (10.35)	1.43 (0.48)	2.5 (1.9–4.0)	1.5 (0.0–3.9)	4.8 (1.6)	4.8 (1.3)	31.5 (10.9)

[a] $n = 2$ on day 1 (means [SEs] are shown), and $n = 4$ at steady state for the 25-mg group; $n = 6$ for all other doses except for the 400-mg group, where n is equal to 5.
[b] One subject in the 400-mg group was not compliant. The day 1 and steady-state pharmacokinetic parameters for this subject were 3.00 and 2.25 μg/ml, respectively, for C_{max}, and 16.24 and 12.55 μg · h/ml, respectively, for AUC_{0-t}. T_{max} was 1.9 h on both occasions.
[c] Mean ratio of individual paired values at steady state to the values on day 1 for all cohorts except the 25-mg cohort, for which the ratio of the cohort mean values is presented.
[d] Two subjects in the 800-mg group took a dose within 12 h of the steady-state intensive pharmacokinetic dose, resulting in higher than expected predose levels. The mean ± SD of the trough concentration (C_{trough}) was 0.88 ± 0.59 μg/ml when the data for these two subjects are excluded.
[e] Median (range).
[f] Calculated from the observed log-linear portion of the post-peak concentration-time curve up to 8 h, not the terminal-phase $t_{1/2}$.
[g] Estimated $t_{1/2\beta}$ from modeling analysis, approximating the terminal-phase half-life.
[h] SS, steady state.
[i] NA, not available.
[j] Data are means (SDs) unless indicated otherwise.

period. This initial distribution-elimination phase had an estimated $t_{1/2}$ that ranged from 2.5 to 5.0 h after the first dose and at steady state across all doses. The presence of a second slower elimination phase was evidenced by the substantial steady-state predose plasma levels, which ranged from 0.15 to 2.65 μg/ml (cohort mean values) for doses ≥100 mg (Table 2).

To approximate the half-life of the unobserved second elimination phase ($t_{1/2\beta}$), a two-compartment model with first-order input and first-order elimination was fitted to individual steady-state plasma concentration-time data from the 100- to 800-mg dose cohorts, with the predose data "flipped over" and considered the 24-h datum points. Mean ± standard error (SE) model estimates were 68.3 ± 8.3 liters for V_1, 0.492 ± 0.083 h^{-1} for K_a, 0.361 ± 0.075 h^{-1} for k_{12}, and 0.052 ± 0.039 h^{-1} for k_{21}, respectively. The mean estimated $t_{1/2\beta}$ ranged from 29.5 to 41.3 h (Table 2). The mean ± standard deviation (SD) model-predicted day 1 and steady-state AUCs from 0 to 8 h were 2.89 ± 0.84 and 4.07 ± 0.67 μg · h/ml, respectively, for the 100-mg dose; 5.48 ± 1.48 and 6.49 ± 1.39 μg · h/ml, respectively, for the 200-mg dose; 10.35 ± 5.64 and 12.93 ± 5.68 μg · h/ml, respectively, for the 400-mg dose, and 19.40 ± 7.06 and 29.89 ± 9.24 μg · h/ml, respectively, for the 800-mg dose. The model-predicted AUCs were therefore in excellent agreement with the observed data presented in Table 2, indicating the adequacy of the modeling analysis. At steady state, the AUC from 0 to 8 h represented approximately 60% of the AUC over the dosing interval (24 h).

Pharmacokinetic-pharmacodynamic relationship. An E_{max} model was used to delineate the relationship between patient serum HBV DNA reduction data (log_{10} scale) at the end of 4 weeks of treatment, a measure of the pharmacodynamic effect of telbivudine, and pharmacokinetic parameters of drug exposure, i.e., C_{max} and AUC_{0-t}, obtained on day 1 and at steady state. The E_{max} model fitted the dose-response data well, in particular, the steady-state C_{max} and AUC_{0-t}, with better precision for model estimates. As summarized in Table 3, model estimates for E_{max} ranging from 3.20 to 3.44 log_{10} were consistent for all four pharmacokinetic parameters examined; estimates of the values of the pharmacokinetic parameters required to produce 50% of E_{max} were 0.06 and 0.12 μg/ml for the day 1 and steady-state C_{max}, respectively, and 0.13 and 0.41 μg · h/ml for the day 1 and steady-state AUC_{0-t}, respectively. The steady-state plasma exposures required to produce a 3-log_{10} or 99.9% reduction in the serum HBV DNA level at week 4 were predicted to be as low as 0.78 μg/ml for C_{max} and 3.36 μg · h/ml for AUC_{0-t}, comparable to the exposures achieved by a dose of 100 mg/day (Table 2). As depicted in Fig. 2, while a more profound virologic response was achieved with higher plasma drug exposure in patients receiving higher telbivudine doses, consistent with its dose-proportional pharmacokinetics, a nearly maximal viral load reduction was obtained with plasma exposures corresponding to telbivudine doses in the 400- to 800-mg range.

DISCUSSION

This dose-escalation study evaluated the safety, pharmacokinetics, and antiviral activity of telbivudine in HBV-infected patients during 4 weeks of oral, once-daily treatment. The results of studies of the safety and antiviral activity of telbivudine, detailed elsewhere (6), demonstrated that telbivudine is well tolerated

TABLE 3. E_{max} model analysis for pharmacokinetic-pharmacodynamic relationship[a]

Parameter	E_{max}		EPK_{50}		R^2
	Estimate	P	Estimate	P	
C_{max}					
Day 1	3.33 ± 0.20	<0.0001	0.06 ± 0.03	0.087	0.9560
Steady state	3.44 ± 0.17	<0.0001	0.12 ± 0.04	0.006	0.9662
AUC_{0-t}					
Day 1	3.20 ± 0.17	<0.0001	0.13 ± 0.09	0.159	0.9538
Steady state	3.36 ± 0.16	<0.0001	0.41 ± 0.14	0.008	0.9663

[a] Model estimates for E_{max} and EPK_{50} are means ± SEs.

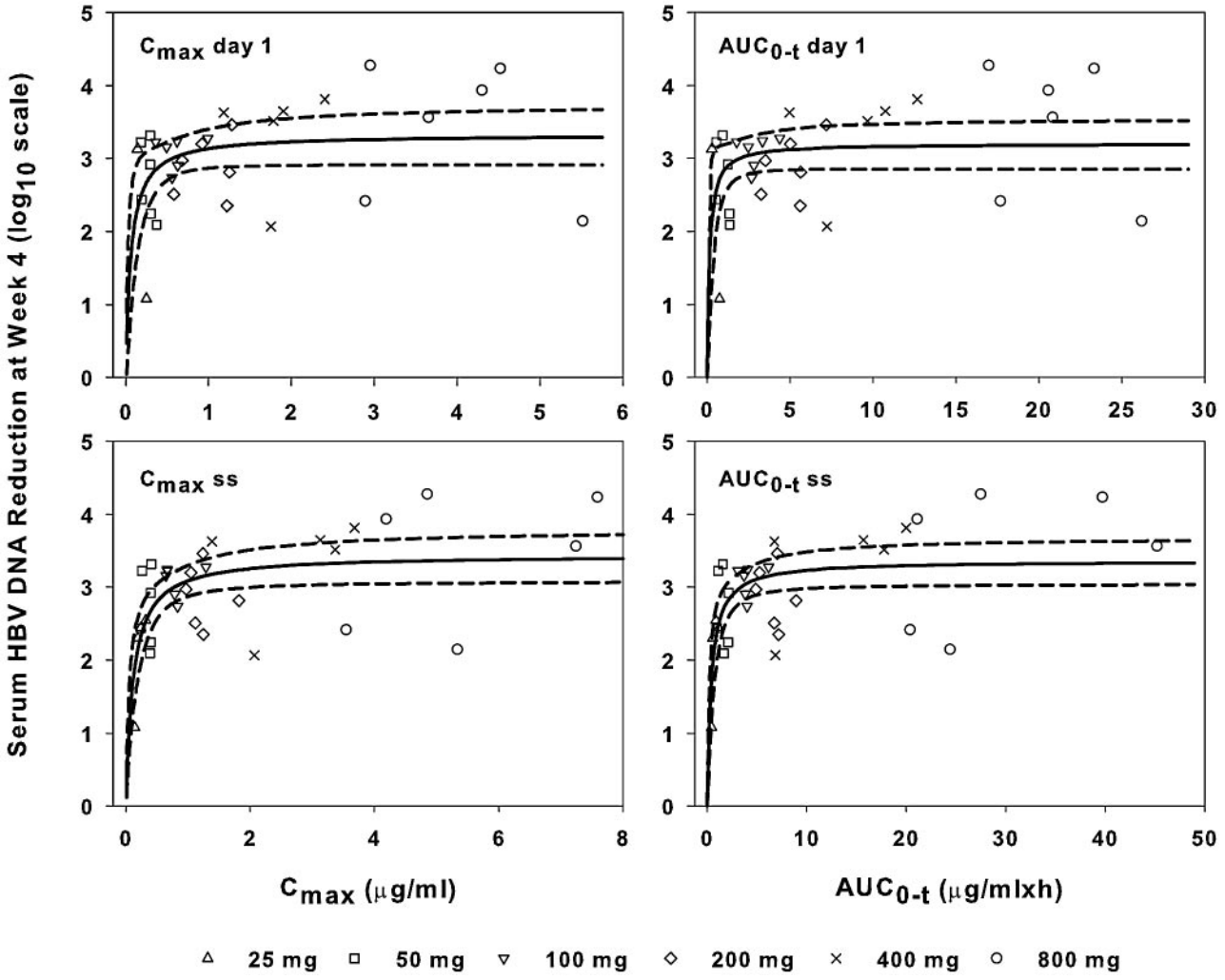

FIG. 2. Plots of serum HBV DNA reduction at week 4 versus single-dose and steady-state telbivudine pharmacokinetic parameters underlying plasma exposure (C_{max} and AUC_{0-t}). Solid and dashed lines, the E_{max} model-predicted response curves and associated 95% confidence intervals, respectively. The symbols represent the dose groups.

without clinically significant adverse events throughout treatment and across all dose levels from 25 to 800 mg/day. Compared to placebo, there were no significant changes in hematologic values and there were no significant abnormalities in clinical laboratory data throughout the trial. Clinical adverse events were all mild, and there was no apparent dose-related or treatment-related pattern in the incidence of adverse events (6). A profound, dose-related virologic response was observed with all doses tested, with serum HBV DNA reductions of 3.5 to 4.0 $\log_{10}$ achieved with doses of 400 to 800 mg/day by the end of 4 weeks of treatment.

The pharmacokinetics of telbivudine were evaluated over a period of 8 h in this first clinical trial conducted with HBV-infected patients. While this sampling interval proved to be inadequate for a full pharmacokinetic characterization of telbivudine, it did capture essential pharmacokinetic parameters, such as C_{max}, T_{max}, and a significant portion of AUC (approximately 60%, based on model-predicted steady-state exposure), therefore allowing assessment of the dose proportionality and the dose-response relationship. The study results indicated that telbivudine is rapidly absorbed after oral dosing, with C_{max} reached within 1 to 3 h. Pharmacokinetic parameters of drug exposure, including C_{max} and AUC_{0-t}, are dose proportional over the dose range studied. While the absolute oral bioavailability of telbivudine remains unknown, the dose-proportional behavior of telbivudine plasma kinetics indicates consistent absorption of the drug across all dose cohorts. In this study, telbivudine was administered with no restriction on food intake. A food-effect study later showed that a high-calorie, high-fat meal had no effect on the pharmacokinetics of telbivudine (unpublished data).

Over the 8-h period, telbivudine exhibits an apparent single-phase decline, with a short observed $t_{1/2}$. This phase later proved to represent an early distribution-elimination phase, as the presence of a second, slower elimination phase was evidenced by the substantial steady-state levels of telbivudine that remained measurable 24 h after dosing (predose trough levels; Fig. 1 and Table 2) in cohorts receiving ≥100 mg/day. A modeling approach was used to characterize this unobserved second elimination phase by flipping the steady-state predose data over to 24 h. Although the lack of datum points between 8 and 24 h prevents the model from generating high-precision esti-

mates of pharmacokinetic parameters, the analysis nevertheless identified the second elimination phase, which had a mean estimated terminal half-life in the range of 29.5 to 41.3 h. The model-predicted values of the AUC from 0 to 8 h were in excellent agreement with the experimental data, indicative of the adequacy of the fitting results. Recent studies with healthy volunteers with intensive sampling up to 168 h postdosing confirmed the existence of this second elimination phase (unpublished data). The second phase starts approximately 16 to 24 h after dosing, with a long observed terminal-phase $t_{1/2}$ of approximately 40 h. This long plasma terminal-phase $t_{1/2}$ of telbivudine is consistent with the long intracellular $t_{1/2}$ (14 h) of the active triphosphate form of the drug observed in HepG2 cells (5). The long half-life of plasma telbivudine and its intracellular triphosphate reflect a sustained exposure of the drug within HBV-infected cells and support the use of once-daily dosing.

Following daily oral dosing, telbivudine accumulated slightly, as evidenced by approximately 15 to 50% increases in C_{max} and AUC_{0-t} at steady state, as well as substantial steady-state predose levels. Of note, the 800-mg cohort exhibited a higher than expected mean predose trough level (2.65 μg/ml) associated with a large SD (2.01 μg/ml). This was apparently caused by the fact that two patients in this group took the steady-state intensive pharmacokinetic dose in less than 12 h of the time that they took the previous day's dose. Unpublished results from several pharmacokinetic studies with healthy volunteers with sampling beyond 24 h showed an accumulation factor of 1.2 to 1.6, based on the ratio of the steady-state AUC from 0 to 24 h to the single-dose AUC from 0 to 24 h, therefore confirming the findings of the present study. The results from those studies further demonstrated that the drug does not accumulate further once steady state is achieved after 5 to 7 days of once-daily treatment (8; unpublished data).

The dose-related virologic response (6) prompted a more detailed evaluation of the telbivudine pharmacokinetic-pharmacodynamic relationship. An E_{max} model was successfully fitted to the individual serum HBV DNA reduction at week 4 versus the pharmacokinetic exposure (C_{max} and AUC_{0-t}) data. The dose-response relationship was more pronounced at steady state, suggesting the importance of maintaining continuous drug exposure, which is ensured by good adherence to treatment, to achieving better antiviral activity. Consistent with the dose-proportional pharmacokinetics of telbivudine, a more profound viral load reduction was observed in patients enrolled in the higher-dose groups. The E_{max} model analyses further indicated that a nearly maximal virologic response was obtained with the plasma exposure achieved with 400- to 800-mg telbivudine doses, and only a minimal incremental virologic response could be gained even with substantially higher doses. Therefore, the results of this exposure parameter-based pharmacokinetic-pharmacodynamic analysis are in full agreement with those of a previous dose-based E_{max} analysis, which demonstrated that a nearly maximal reduction of circulating serum HBV DNA (~3.5 to 4.0 $\log_{10}$) was obtained by week 4 with telbivudine doses between 400 to 800 mg. Based on these findings, telbivudine doses appropriate for further clinical evaluation were selected, and doses higher than 800 mg/day were not evaluated (6), despite the excellent safety profile and dose-proportional pharmacokinetics.

In summary, this clinical evaluation demonstrates that telbivudine exhibits dose-proportional pharmacokinetics and exposure-dependent pharmacodynamics and is well tolerated by patients with chronic HBV infection (6). This favorable profile of telbivudine supports ongoing phase III trials for evaluation of the safety and efficacy of longer-term treatment with telbivudine in patients with chronic hepatitis B.

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REFERENCES

1. **Bridges, E., E. Cretton-Scott, X. J. Zhou, A. Juodawlkis, G. Gosselin, and M. Bryant.** 2001. The anti-HBV agent β-L-thymidine (LdT) exhibits no observable effects in chronic developmental toxicity studies, p. 84–85, abstr. 85. Proc., Frontiers in Drug Development for Viral Hepatitis.
2. **Bridges, E. G., M. L. Bryant, L. Placidi, A. Faraj, A. G. Loi, C. Pierra, D. Dukhan, G. Gosselin, J. L. Imbach, B. Hernández, A. Juodawlkis, B. Tennant, E. Cretton-Scott, and J. P. Sommadossi.** 2001. Toxicological study of the anti-HBV agent β-L-2′-deoxythymidine, p. 263–270. *In* R. F. Schinazi, J. P. Sommadossi, and C. M. Rice (ed.), Frontiers in viral hepatitis. Elsevier B.V., Amsterdam, The Netherlands.
3. **Bryant, M. L., E. G. Bridges, L. Placidi, A. Faraj, A. G. Loi, C. Pierra, D. Dukhan, G. Gosselin, J. L. Imbach, B. Hernández, A. Juodawlkis, B. Tennant, B. Korba, P. Cote, P. Marion, E. Cretton-Scott, R. F. Schinazi, and J. P. Sommadossi.** 2001. Antiviral L-nucleosides specific for hepatitis B virus infection. Antimicrob. Agents Chemother. **45:**229–235.
4. **Cretton-Scott, E., X. J. Zhou, E. G. Bridges, B. Tennant, A. Juodawlkis, G. Gosselin, J. L. Imbach, C. Pierra, D. Dukhan, R. F. Schinazi, J. P. Sommadossi, and M. Bryant.** 1999. Pharmacokinetics of β-L-thymidine and β-L-2′-deoxycytidine in woodchucks and monkeys. Antivir. Ther. **4:**A124.
5. **Hernandez-Santiago, B., L. Placidi, E. Cretton-Scott, A. Faraj, E. Bridges, M. L. Bryant, J. Rodriguez-Orengo, J. L. Imbach, G. Gosselin, C. Pierra, D. Dukhan, and J. P. Sommadossi.** 2001. Pharmacology of β-L-2′-deoxythymidine and β-L-2′-deoxycydine in HepG2 cells and primary human hepatocytes: relevance to chemotherapeutic efficacy against hepatitis B virus. Antimicrob. Agents Chemother. **46:**1728–1733.
6. **Lai, C. L., S. G. Lim, N. A. Brown, X. J. Zhou, D. M. Lloyd, Y. M. Lee, M. F. Yuen, G. C. Chao, and Maureen W. Myers.** 2004. A dose-finding study of once-daily oral telbivudine (LdT) in HBeAg-positive patients with chronic hepatitis B virus infection. Hepatology **40:**719–726.
7. **Lai, C. L., N. Leung, E. K. Teo, M. Tong, F. Wong, H. W. Hann, S. Han, T. Poynard, M. Myers, G. Chao, D. Lloyd, and N. A. Brown.** 2005. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology **129:**528–536.
8. **Zhou, X. J., S. G. Lim, C. L. Lai, P. F. Lam, D. M. Pow, N. A. Brown, and M. W. Myers.** 2001. Pharmacokinetics of β-L-deoxythymidine (LdT) in healthy subjects and patients with chronic hepatitis B virus infection, p. 92, abstr. 100. Proc., Frontiers in Drug Development for Viral Hepatitis.