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Nonclinical Safety Profile of Telbivudine, a Novel Potent Antiviral Agent for Treatment of Hepatitis B[▽]

Edward G. Bridges,† Jules R. Selden,* and Shouqi Luo

Idenix Pharmaceuticals, Inc., One Kendall Square, Cambridge, Massachusetts 02139

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Telbivudine is a novel nucleoside drug recently approved for the treatment of patients with chronic hepatitis B. Its nonclinical safety was evaluated in a comprehensive program of studies, including safety pharmacology, acute and chronic toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. There were no test article-related effects observed in an in vitro hERG assay or in a core battery of safety pharmacology studies (central nervous system, respiratory, and cardiovascular safety pharmacology studies). Telbivudine was well tolerated in rats and in monkeys following single oral doses up to 2,000 mg/kg/day. Except for equivocal axonopathic findings in monkeys and occasional incidences of emesis, soft feces, and minor changes in body weight and food consumption, there was no target organ toxicity observed in mice, rats, or monkeys following oral administration for up to 3, 6, or 9 months, respectively, at doses up to 3,000 mg/kg/day. Axonopathy in the sciatic nerves and in the spinal cords of monkeys dosed at 1,000 mg/kg/day observed in a 9-month study was considered equivocal, as the role of telbivudine in the injury could not be determined. Slightly higher incidences of abortion and premature delivery observed in rabbits dosed at 1,000 mg/kg/day were considered secondary to maternal toxicity. There was no evidence of genotoxicity or carcinogenicity. These results suggest that telbivudine has a favorable safety profile and support its use in patients with chronic compensated hepatitis B viral infection.

Telbivudine (β-L-2′-deoxythymidine) is the unmodified β-L enantiomer of the naturally occurring nucleoside thymidine (Fig. 1). The chemical name is 1-[(2*S*,4*R*,5*S*)-4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl]-5-methyl-1*H*-pyrimidine-2,4-dione, or 1-(2-deoxy-β-L-ribofuranosyl)-5-methyluracil. Its molecular formula is $C_{10}H_{14}N_2O_5$ with a formula weight of 242 g/mol.

Nucleoside/nucleotide analogs have been proven to be effective for the treatment of hepatitis B virus (HBV) and human immunodeficiency virus. To date, four nucleoside/nucleotide analogs, lamivudine, adefovir dipivoxil, entecavir, and telbivudine, have been approved by the United States Food and Drug Administration for the treatment of HBV (16, 27). These agents vary with respect to antiviral and clinical efficacy, resistance profiles, and tolerability and safety (17). A major safety concern for this class of drugs is mitochondrial toxicity, which is manifested as hepatic failure, nephrotoxicity, pancreatitis, neuropathy, myopathy, and lactic acidosis (1, 2, 3, 5, 6, 8, 15, 18, 19, 22, 24, 29). Furthermore, emergence of drug-resistant viruses in patients undergoing long-term maintenance therapy with these drugs can result in diminished drug efficacies (9, 30).

Telbivudine shows potent, selective, and specific antiviral activity against HBV and other hepadnaviruses (4, 14). Telbivudine is phosphorylated by intracellular thymidine kinases to the active triphosphate form, which has an intracellular half-life of 14 h (12, 25). Telbivudine 5′-triphosphate inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, dTTP. Incorporation of telbivudine 5′-triphosphate into viral DNA causes DNA chain termination, resulting in inhibition of HBV replication (4, 12).

In hepatoma cell culture assays, telbivudine inhibits HBV production with a mean 50% inhibitory concentration (IC_{50}) ranging from 0.05 to 0.65 μM, depending on the cell type, growth conditions, and the assay used. Telbivudine is active against duck HBV in primary duck hepatocytes (IC_{50} = 0.18 μM). In the woodchuck model of chronic hepatitis, serum levels of woodchuck hepatitis virus DNA decreased by 10^8 genome equivalents per milliliter after 4 to 6 weeks of treatment with 10 mg of telbivudine/kg/day. In enzymatic assays, telbivudine 5′-triphosphate inhibits the woodchuck hepatitis virus DNA polymerase with an IC_{50} value of 0.24 μM (4, 25).

Telbivudine is relatively nontoxic in a variety of cell types. The 50% cytotoxic concentration is >2,000 μM in cultured human hepatoma cells, >200 μM in human peripheral blood mononuclear cells (25), and >400 μM in human foreskin fibroblasts (unpublished data). Granulocyte-macrophage progenitors (CFU-granulocyte-macrophage) and erythroid progenitors (burst-forming units-erythroid) exposed to telbivudine in clonogenic assays, which routinely detect the cellular toxicity of zidovudine (AZT), are not affected at 10 μM (25).

This article reports the results of the extensive nonclinical safety program for telbivudine designed to characterize its toxicity profile and to identify biomarkers for toxicity monitoring in patients.

MATERIALS AND METHODS

Compound. Telbivudine was manufactured at Microbiologica Quimica e Farmaceutica (Rio de Janeiro, Brazil) in compliance with concurrent good manufacture practice. The purity was ≥99.1%. Telbivudine was formulated in 0.5% (wt/vol) carboxymethylcellulose and administered to mice, rats, rabbits, or monkeys at a dose volume of 5 or 10 ml/kg.

* Corresponding author. Mailing address: Idenix Pharmaceuticals, Inc., 60 Hampshire Street, Cambridge, MA 02139. Phone: (617) 995-9800. Fax: (617) 224-4350. E-mail: selden.jules@idenix.com.

† Present address: West Virginia School of Osteopathic Medicine, 400 North Lee Street, Lewisburg, WV 24901.

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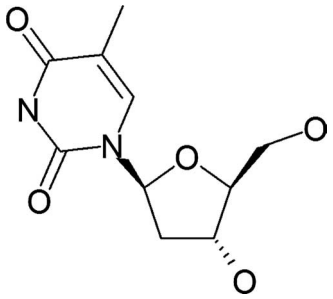

FIG. 1. Structure of telbivudine.

Animals. CD-1 and CB6F1 mice were obtained from Charles River Laboratories (Raleigh, NC). CB6F1-Tg *ras*H2 mice were obtained from the Central Institute for Experimental Animals (Kanagawa, Japan). Sprague-Dawley rats [Crl:CD(SD)IGS BR] were obtained from Charles River Laboratories (Raleigh, NC). Female New Zealand White rabbits were obtained from Covance Research Products, Inc. (Denver, PA). Cynomolgus monkeys (*Macaca fascicularis*) were obtained from Charles River Primates (Houston, TX), Covance Research Products, Inc. (Alice, TX), Primate Products, Inc. (Miami, FL), or Guangdong Scientific Instruments and Materials Import and Export Corporation (Guangdong, China). Animal studies were conducted at SNBL USA Ltd. (Everett, WA), Charles River Laboratories (Horsham, PA, and Worcester, MA), or Covance Laboratories Inc. (Vienna, VA) according to good laboratory practice or accepted scientific and industrial standards.

Safety pharmacology studies. Safety pharmacology studies were performed to assess the potential for telbivudine to produce any adverse pharmacological effects. Telbivudine was administered to conscious telemeterized male cynomolgus monkeys (4 males/group) on four separate occasions (4-by-4 Latin Square design) at doses of 0, 250, 750, or 2,000 mg/kg by oral gavage to evaluate the effects on the function of the respiratory and cardiovascular systems. In the central nervous system safety study, single doses of 0, 150, 500 and 1,000 mg/kg of telbivudine were orally administered to 8 rats/sex/group.

The cardiac hERG (human *ether-à-go-go*-related gene) channel is a potassium channel responsible for rapid delayed rectifier outward currents (I_{Kr}). Inhibition of I_{Kr} is the most common cause of cardiac action potential prolongation by noncardiac drugs, leading to prolongation of the QT interval and an associated ventricular arrhythmia disorder, torsade de pointes. The effect of telbivudine on the hERG current was determined at concentrations of 10, 100, 1,000, and 10,000 μM in human embryonic kidney cells (HEK293) stably transfected with a human ERG gene (3 to 4 cells/group).

Acute toxicity studies. Four groups of 5 rats/sex/group were orally administered with a single dose of vehicle (0 mg/kg) or telbivudine at 500, 1,000, or 2,000 mg/kg. One group of 2 monkeys/sex received a starting dose of 20 mg/kg of telbivudine. As no overt toxicity was observed, the monkeys received escalating doses of 100, 500, 1,000, and finally 2,000 mg/kg with a washout period of 3 to 4 days between doses. Rats were observed for 14 days, whereas monkeys were observed for 3 days following the last dose administration. Acute toxicity was evaluated based on clinical observations, body weights, clinical pathology, and macroscopic pathology.

Repeat-dose toxicity studies. The multiple-dose nonclinical safety studies conducted in support of the telbivudine program are listed in Table 1. Telbivudine was administered by oral gavage to mice for 28 days or 13 weeks at doses up to 3,000 mg/kg/day and to rats and monkeys for 28 days or 3, 6 (rats only), or 9 (monkeys only) months at doses up to 2,000 mg/kg/day. Repeat-dose toxicity was evaluated based on mortality; clinical observations; body weight; food consumption; ophthalmology; clinical pathology, including hematology, coagulation, and chemistry; and/or macroscopic and microscopic pathology. Electrocardiogram and vital signs such as blood pressure, heart rate, and body temperature were also collected in the monkey studies for toxicity assessment.

Reproductive and developmental toxicity studies. Five studies were conducted to evaluate the effects of telbivudine on fertility in male and female rats, on embryo-fetal development in female rats and New Zealand White rabbits, and on multiple generation development in male and female rats (Table 2).

In a combined rat fertility and embryo-fetal development study (combined segments I and II), male rats (25 animals/group) were orally administered telbivudine at doses of 0, 100, 500, or 1,000 mg/kg/day, starting 28 days prior to cohabitation and continuing through the day before the completion of cohabitation. Female rats (25 animals/group) were dosed starting 15 days prior to cohabitation and continuing through gestation day 17. Male rats were sacrificed at the completion of cohabitation, whereas females were sacrificed on gestation day 21.

In a follow-up male fertility study (segment I), male rats (25 to 26 animals/group) were dosed with telbivudine at doses of 0, 1,000, or 2,000 mg/kg/day, starting 28 days prior to cohabitation and continuing through the day before the completion of cohabitation. Male rats were sacrificed at the completion of cohabitation, whereas untreated cohabitated females were sacrificed on gestation day 13. In another follow-up female fertility study (segment I), female rats (25 animals/group) were dosed with telbivudine at doses of 0 or 2,000 mg/kg/day, starting 15 days prior to cohabitation and continuing through gestation day 7. Females were sacrificed on gestation day 13, whereas untreated cohabitated male rats were sacrificed at the completion of cohabitation.

Male fertility toxicity was evaluated based on mating and impregnating rate; sperm count, mobility, and morphology; and microscopic examinations of testes and epididymides. Female fertility was evaluated based on estrous cycling, mating and pregnancy rate, number of corpora lutea, number and distribution of implantation sites, early resorption, and microscopic examination of ovaries. Fetotoxicity and teratologic potential were evaluated based on mid and late resorptions; the numbers of live and dead fetuses; external sex; body weight; and gross external alterations and microscopic internal alterations, such as major malformations, minor external visceral and skeletal anomalies, and common skeletal variants.

In an embryo-fetal development study (segment II) in female New Zealand White rabbits, four groups of 20 artificially inseminated New Zealand hybrid rabbits were orally administered telbivudine at doses of 0, 50, 250, or 1,000 mg/kg/day on day 6 through day 18 postinsemination. The does were caesarean sectioned on day 29 postinsemination. The numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were recorded. Fetuses were examined for external sex, body weight, gross external alterations, and microscopic internal alterations. Pregnancy rates and pre- and postimplantation losses were calculated.

In a multiple generation development study (segment III) in male and female rats, telbivudine was administered by oral gavage at doses of 0, 100, 250, or 1,000 mg/kg/day to female Crl:CD(SD)IGS BR rats (25 animals/group) on gestation day 7 through postpartum day (PD) 20. Females were allowed to deliver and maintain their progeny until PD 21. On PD 21, dams were sacrificed and implantation sites were counted. Pups not scheduled for continued evaluation were sacrificed on PD 21 and examined for gross lesions and microscopic changes on selected tissues. From the surviving pups, one rat/sex/group of the first offspring (F_1) was evaluated for passive avoidance tests for learning and short-term and

TABLE 1. Repeat-dose oral toxicity studies with telbivudine in mice, rats, and monkeys

Study type and duration	Species	Doses (mg/kg/day)	Animal assignment
4-wk toxicity with toxicokinetics	Mouse (CB6F1/Crl: BR)	0, 500, 1,000, 2,000	10/sex/group
13-wk toxicity with toxicokinetics	Mouse [Crl:CD-1(ICR) BR]	0, 500, 1,000, 3,000	10/sex/group
28-day toxicity with toxicokinetics	Rat [Crl:CD(SD) BR]	0, 500, 1,000, 2,000	10/sex/group
6-mo toxicity with 3-mo interim sacrifice	Rat [Crl:CD(SD) BR]	0, 250, 500, 1,000	10/sex/group[a]
28-day toxicity with toxicokinetics	Monkey (cynomolgus)	0, 500, 1,000, 2,000	10/sex/group
9-mo toxicity with 3-mo interim sacrifice	Monkey (cynomolgus)	0, 250, 500, 1,000	3–4/sex/group[b]

[a] An additional 5 rats/sex were assigned to control and high-dose groups to serve as a 1-month recovery subgroup.
[b] Three monkeys/sex/group were scheduled to be sacrificed following 3 months of dosing and 4 monkeys/sex/group were sacrificed following 9 months of dosing. An additional 2 monkeys/sex were assigned to control and high-dose groups to serve as a 2-month recovery subgroup.

TABLE 2. Reproductive and developmental toxicity studies with telbivudine in rats and rabbits

Study type and duration	Species	Dose (mg/kg/day)	Animal assignment	Dosing period
Fertility, early embryonic development, embryo-fetal developmental (segments I and II)	Rat [Crl:CD(SD)IGS BR]	0, 100, 500, 1,000	25/sex/group	Males, 28 days prior to cohabitation through the day before completion of cohabitation; females, 15 days prior to cohabitation through gestation day 17[a]
Fertility, male (segment I)	Rat [Crl:CD(SD)IGS BR]	0, 1,000, 2,000	25–26/sex/group[b]	Males, 28 days prior to cohabitation through the day before completion of cohabitation[a]
Fertility, female (segment I)	Rat [Crl:CD(SD)IGS BR]	0, 2,000	25/sex/group[c]	Females, 15 days prior to cohabitation through gestation day 7[a]
Embryo-fetal development (segment II)	Rabbit [Hra (NZW) SPF]	0, 50, 250, 1,000	20 females/group	Gestation days 6 through 18
Multiple generation development (segment III)	Rat [Crl:CD(SD)IGS BR]	0, 100, 250, 1,000	25 females/group	Gestation day 7 through PD 20

[a] Males and females were cohabitated (1:1) for a maximal period of 21 days. Females not mated within the first 14 days of cohabitation were assigned an alternate male rat from the same dose group that had mated and remained in cohabitation for a maximum of seven additional days. Likewise, males not mated within the first 14 days of cohabitation were assigned an alternate female rat and remained in cohabitation for a maximum of three additional days.
[b] The 25 to 26 females/group were used for mating only.
[c] The 25 males/group were used for mating only.

long-term retention starting from PD 24 ± 1. Females were examined for the age of vaginal patency starting from PD 28. Males were examined for the age of preputial separation starting from PD 39. On approximately PD 70, one rat/sex/litter/group was evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory. At the age of 90 days, one rat/sex/group was cohabitated for a maximum of 21 days. Once females were observed to have spermatozoa in their vaginal smears or a copulatory plug, they were housed independently. F_1 males were sacrificed at the end of cohabitation, and the testes and epididymides were weighed. F_1 females were sacrificed on gestation day 21 and evaluated in like manner to the F_0 females.

Genetic toxicology studies. Three in vitro assays and one in vivo test were performed to assess the genotoxic potential for telbivudine. Reverse mutation potential was assayed with *Salmonella enterica* serovar Typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA. The mutagenic potential was tested with in vitro chromosomal aberration assays in Chinese hamster ovary cells and in human peripheral blood lymphocytes. The in vitro assays were performed at concentrations up to 5,000 μg/plate (bacterial mutation), 2,442 μg/ml (human peripheral blood lymphocytes), or 5,000 μg/ml (Chinese hamster ovary cells) with or without metabolic activation (S9). An in vivo micronucleus test was performed in CD-1 mice given single oral doses of 0, 500, 1,000, or 2,000 mg/kg. The numbers of micronuclei in bone marrow preparations obtained at 24 and 48 h postdose were determined. For all in vitro assays and in vivo tests, telbivudine was prepared as a solution in dimethyl sulfoxide and appropriate negative and positive controls were used.

Carcinogenicity studies. The carcinogenic potential of telbivudine was tested in two bioassays. In a 6-month carcinogenicity study in CB6F1-Tg *ras*H2 mice, telbivudine was administered once daily by oral gavage to groups of 25 mice/sex/group at doses of 0, 500, 1,000, or 2,000 mg/kg/day. In addition, an additional group of 25 mice/sex received a known carcinogen, *N*-methyl-*N*-nitrosourea (MNU), intraperitoneally at a dose of 75 mg/kg on day 1 as a positive control. In a traditional 2-year carcinogenicity study in Sprague-Dawley rats [Crl:CD (SD)IGS BR], telbivudine was administered to groups of 65 rats/sex/group by oral gavage at doses of 0, 500, 1,000, or 2,000 mg/kg/day. As mortality reached 38% (25/65) in both male and female rats dosed at 2,000 mg/kg/day at week 85, dosing of this group was discontinued after the completion of 85 weeks. The remaining animals continued to receive dosing until the completion of 95 weeks of dosing. Animals were sacrificed at weeks 95 (group 2 males), 96 (groups 1, 3, and 4 males), and 97 (all females). Toxicity and carcinogenicity were evaluated based on survival; clinical observations, including grossly visible and/or palpable masses; body weight; food consumption; clinical pathology; and macroscopic and microscopic pathology.

Statistical analyses. For continuous data such as body weight, food consumption, clinical pathology, and organ weight, Bartlett's test or Levene's test was used to test for variance homogeneity. In the case of heterogeneity of variance at $P \leq 0.05$, rank transformation was used to stabilize the variance. One-way analysis of variance was used to analyze data. When the result was significant ($P \leq 0.05$), Dunnett's test was used for comparisons between dosed and control groups. Group comparisons (dosed groups versus control groups) were evaluated at the 5.0% two-tailed probability level.

For data that exhibited heterogeneity after transformations, a Kruskal-Wallis test was applied. If the result was significant, a nonparametric Dunn's test was employed for comparisons between dosed and control groups.

Histopathology data were analyzed for incidence of neoplastic and nonneoplastic lesions using Fisher's exact test to compare dosed groups and positive control groups versus the vehicle control groups. The incidence of common background findings was compared with controls and published values from different laboratories (26).

RESULTS

Safety pharmacology studies. Telbivudine had no effects on the respiratory and cardiovascular functions as observed in the monkey study following the administration of single doses up to 2,000 mg/kg and no effects on the central nervous system function in the rat study following the administration of single doses up to 1,000 mg/kg. Results from the hERG assay showed that hERG currents were not affected by telbivudine at concentrations up to 10,000 μM in HEK293 cells stably transfected with a human ERG gene.

Acute toxicity studies. No test article-related changes in clinical signs, body weight, clinical pathology, organ weight, or macroscopic pathology were found in rats following a single oral administration of 2,000 mg/kg/day. In the monkeys, telbivudine was well tolerated following the administration of escalating doses up to 2,000 mg/kg/day. No overt toxicity or adverse effects were observed.

Repeat-dose toxicity studies. In the 13-week repeat-dose toxicity study in mice, there was no evidence for systemic toxicity from telbivudine at any dose tested. Four mice (one male dosed at 1,000 mg/kg/day, plus two males and one female dosed at 3,000 mg/kg/day) were found dead on study days 88 and 89. The lungs of these mice were congested, suggesting possible aspiration of the test article. Mean body weights were occasionally higher in females of all dosed groups and in males of mid- and high-dose groups. However, there was no statistical correlate between body weight and food consumption. The mean absolute liver weight in the females dosed at 1,000 mg/kg/day was higher than in controls,

TABLE 3. Incidence of axonopathy in the sciatic nerves and spinal cords in monkeys following once daily oral administration for 9 months

Site	Interval	Incidence of axonopathy by sex and dose (mg/kg/day)[a]							
		Males				Females			
		0	500	1,000	2,000	0	500	1,000	2,000
Sciatic nerve	3 mo	0/3	0/3	0/3	1/3 (G1)	0/3	0/3	0/3	1/3 (G1); 1/3 (G2)
	9 mo	1/4 (G1)	2/4 (G1)	0/4	0/4	2/4 (G1)	2/4 (G1)	0/4	3/4 (G1)
	Recovery	0/2	NA	NA	1/2 (G1)	0/2	NA	NA	0/2
Spinal cord	3 mo	0/3	2/3 (G1)	1/3 (G1)	1/3 (G1)	1/3 (G1); 1/3 (G2)	1/3 (G1)	1/3 (G1); 1/3 (G2)	0/3
	9 mo	0/4	1/4 (G1)	1/4 (G1)	2/4 (G1); 1/4 (G2)	2/4 (G1)	0/4	1/4 (G1)	1/4 (G1)
	Recovery	0/2	NA	NA	0/2	0/2	NA	NA	0/2

[a] Abbreviations: NA, not applicable; G1, grade 1, minimal, one fiber was affected in the tissue sections examined; G2, grade 2, mild, two to four fibers were affected in the tissue sections examined.

but mean weights relative to body or brain weight were not different from controls. Microscopic findings were consistent with changes common to Crl:CD-1(ICR) BR mice of 21 weeks of age. The no-observed-adverse-effect level (NOAEL) was reported to be 3,000 mg/kg/day.

In the 28-day repeat-dose toxicity study in mice, statistically significant increases in leukocyte and lymphocyte counts were observed in males given 2,000 mg/kg/day. Slight but statistically significant increases in red blood cell counts, hemoglobin concentrations, and hematocrit values were observed in females given 2,000 mg/kg/day. These changes were not considered to be test article related. Moreover, they were not observed in the 13-week study.

In the 6-month repeat-dose toxicity study with 3-month interim sacrifice in rats, six rats in total were found dead (one control male, two males dosed at 500 mg/kg/day, and two females dosed at 1,000 mg/kg/day) or sacrificed for humane reasons (one male dosed at 1,000 mg/kg/day). Except for one male dosed at 500 mg/kg/day that was found dead on day 161 and whose cause of death was undetermined, the cause of death for the remaining four rats was considered to be gavage related. The male dosed at 1,000 mg/kg/day that was sacrificed for humane reasons on study day 133 was diagnosed with widespread lymphoma, a common spontaneous tumor. None of these deaths was attributed to telbivudine. Treatment had no adverse effects on food consumption, but some dosed rats had intervals of increased food intake. This transient increased appetite was not considered evidence for toxicity from telbivudine. Ophthalmic examinations of rats in all dose groups did not identify any treatment-related changes; there were some age-related changes. Hematology data did not indicate toxicity from telbivudine after 92 days (3 months), 176 days (6 months), or 204 days (1-month recovery). There were no conclusive findings from the clinical chemistry data to indicate evidence for toxicity at any interval. Mean absolute and relative organ weights were somewhat variable but failed to demonstrate a clear pattern for toxicity from telbivudine. No macroscopic or microscopic morphological changes were associated with telbivudine exposure. The NOAEL was reported to be 1,000 mg/kg/day.

In the 28-day repeat-dose toxicity study in rats, males dosed at 1,000 and 2,000 mg/kg/day consumed more food at select intervals, but these increases were not judged to represent toxic effects. A statistically significant decrease in mean absolute neutrophil counts was observed in males given 2,000 mg/kg/day. However, this finding was not considered to be of biological significance, as the individual values were still within the normal range of the testing facility for this parameter. The relationship of telbivudine to higher adrenal weights in some female rats was inconclusive. Both the macroscopic and microscopic pathology examinations were unremarkable.

In the 9-month repeat-dose toxicity study in monkeys with 3-month interim sacrifice, female monkeys dosed with telbivudine tended to experience soft stools and emesis more often than control females in a general dose-related manner. However, similar findings in males failed to indicate a role for telbivudine, as controls were equally affected. Soft stools seemed to diminish during the recovery phase. After 3 months of treatment, a lower mean serum alkaline phosphatase level was seen in males dosed at 250 or 1,000 mg/kg/day, primarily attributable to several high values in control males. At 9 months, mean serum aspartate aminotransferase was higher and alkaline phosphatase was lower in all treated male monkeys, likely due to artifacts in control values.

Minimal to mild degeneration was observed in the sciatic nerve sections from two high-dose female monkeys at the 3-month interim sacrifice and in one high-dose female monkey at the 9-month necropsy. Tissue sections of the sciatic nerves and the cervical, thoracic, and lumbar spinal cords of all animals were examined by a second study pathologist and an independent peer review neuropathologist. The degenerative changes were consistent with axonopathy, given the formation of digestion chambers with macrophages and axonal spheroid formation. Compared with the controls, axonopathy appeared to be more frequently observed in the sciatic nerves from females and in the spinal cords from males dosed at 1,000 mg/kg/day (Table 3). The axonopathic changes noted in the sciatic nerves were consistent with nerve fibers undergoing Wallerian degenerative change. Perineuritis, epineuritis, and/or myodegeneration were occasionally noted in the study, but with no relationship to dose group. A scar characterized by perineural disruption and focal fibrosis was noted by the pathologists in one monkey. These findings indicated that at least some of the axonopathic changes might have been secondary to local nerve trauma rather than test article related. However, the increase in incidence of axonopathic changes in the sciatic nerves of high-dose female monkeys was confounding. After a thorough reexamination and careful evaluation, it was concluded that the role of telbivudine in the pathogenesis of the axonopathic changes was

equivocal. Using a conservative approach, the NOAEL was considered to be 500 mg/kg/day.

In the 28-day repeat-dose toxicity study in monkeys, emesis was noted in eight monkeys at 16 instances, mostly in telbivudine-dosed monkeys. There was a high incidence of soft feces across all dose groups, including controls, and this finding was more pronounced in the 2,000 mg/kg/day dose group.

Developmental and reproductive toxicity studies. (i) Male and female fertility. In the combined segment I and II study in rats, there were no test article-related changes in male or female fertility following the administration of telbivudine. However, the pregnancy rate was 76% in rats dosed at 500 mg/kg/day and 72% in rats dosed at 1,000 mg/kg/day; these rates were slightly lower than that of the control group (92%) but still remained within the historical control range of the testing facility. In order to confirm if the lower fertility was telbivudine related, two more fertility studies were conducted, one in males only and the other in females only at doses up to 2,000 mg/kg/day. The results from both studies confirmed that telbivudine had no effects on the pregnancy rate or on the male and female fertility.

(ii) Embryo-fetal development. There were no changes in embryo-fetal development following the administration of telbivudine in the combined segment I and II study in rats or in the segment II study in rabbits. In the segment II study in rabbits, one doe dosed at 1,000 mg/kg/day aborted on day 28 postinsemination and two does dosed at 1,000 mg/kg/day delivered prematurely on gestation day 29. Signs of maternal toxicity included reduced body weight gain, reduced food consumption, and abnormal feces at 1,000 mg/kg/day.

(iii) Multiple generation development. In the multiple generation development study in rats, telbivudine did not produce any adverse effects at doses up to 1,000 mg/kg/day. All pregnant dams (F_0) delivered litters without evidence of dystocia. There were no telbivudine-related clinical observations or pathology findings. There were no telbivudine-related findings in behavior, sexual maturation, or fertility in the F_1 offspring. Furthermore, in utero examination findings were normal in second-generation offspring (F_2). The NOAEL was reported to be 1,000 mg/kg/day for the dams and the fetuses of both F_1 and F_2.

Genetic toxicology studies. A standard battery of genotoxicity testing evaluated the potential effects of telbivudine. The microbial mutagenesis assay was negative in all five bacterial strains tested at doses up to 5,000 μg/plate. In two in vitro chromosomal aberration assays with cultured Chinese hamster ovary cells (up to 5,000 μg/ml) and with cultured human peripheral blood lymphocytes (up to 2,422 μg/ml) telbivudine showed no evidence of clastogenicity. In addition, no evidence of chromosome damage occurred in a mouse micronucleus test following single oral doses up to 2,000 mg/kg.

Carcinogenicity studies. In the 6-month carcinogenicity study in CB6F1-Tg *ras*H2 mice, survival rates were 92% for males and 80% for females in the vehicle control group, 20% for males and 36% for females in the positive control group, and 92% to 100% in the telbivudine-dosed groups. Neoplasms observed in the MNU-dosed mice included hemic-lymphatic lymphosarcoma, squamous cell carcinoma and sarcoma of the skin, pulmonary alveolar/bronchial adenoma, splenic hemangiosarcoma, squamous cell carcinoma of the forestomach, and intestinal adenoma/adenocarcinoma, indicating the validity of this carcinogenic animal model. Neoplasms observed in the 2,000 mg/kg/day dose group included thymic lymphosarcoma in one male, hepatocellular adenoma in one male, pulmonary alveolar/bronchial adenoma in one male, papilloma in the forestomach in one male, and splenic hemangiosarcoma in one male and one female. The incidences of these neoplasms were within historical control ranges of the testing facility for this transgenic strain and were not significantly different from concurrent controls. Thus, daily oral administration of telbivudine at doses up to 2,000 mg/kg/day for 26 weeks did not result in macroscopic or microscopic neoplastic or nonneoplastic changes in CB6F1-Tg *ras*H2 CB6F1 mice.

In the traditional 2-year carcinogenicity study in Sprague-Dawley rats, survival rates were 38% (25/65 rats) in males and females dosed at 2,000 mg/kg/day at week 85 (Table 4). Survival rates ranged from 42% to 55% in other groups. Pituitary adenomas in males and females and mammary carcinomas and fibroadenomas in females were the tumors that were identified as the most-frequent causes of deaths (Table 4). These tumors were present in all test groups, including controls, and there was no evidence for increased tumor-related mortality in the telbivudine-dosed rats (Table 5).

There were no statistically significant trends in the incidence of neoplasms. However, the incidence of acinar cell adenomas of the pancreas was slightly increased in males dosed at 2,000 mg/kg/day (Table 5). In the females, slightly increased incidences of benign pheochromocytomas of the adrenal medulla and fibroadenomas of the mammary gland were observed at 2,000 mg/kg/day (Table 5). Since the incidence of these common tumors was within the historical range for Sprague-Dawley rats and there were no statistically significant trends, these increases were not considered telbivudine related.

Additionally, there were no statistically significant trends noted in the analysis of the incidence of nonneoplastic lesions in rats. Chronic progressive nephropathy (CPN) was observed in almost all animals, including controls. The incidence was not different from controls. There was a slightly higher incidence of nephropathy-related deaths in the male rats dosed at 1,000 and 2,000 mg/kg/day and in the female rats dosed at 2,000 mg/kg/day compared to the incidence in concurrent male and female controls, suggesting that telbivudine might exacerbate this age-related disease. However, there was no evidence for increased severity of nephropathy based on mean severity scores/group nor an increased overall incidence of nephropathy in any treated group. Because CPN is a rodent-specific disease, the exacerbation of CPN by telbivudine can be regarded as having no relevance for extrapolation in human risk assessment.

Steady-state exposure and animal-to-human exposure multiples. The steady-state exposure to telbivudine at the NOAEL and animal-to-human exposure multiples in mice, rats, rabbits, and monkeys are presented in Table 6. Human steady-state maximum concentration of drug in serum (C_{max}) (3.4 μg/ml) and the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) (27.5 μg · h/ml) were obtained from healthy subjects following 7 days of once daily oral administration of 600 mg/day of telbivudine, the approved dose for patients with chronic HBV infection (31). In repeat-dose toxicity studies, the steady-state animal-to-human exposure multiples at the NOAEL were 41, 11, and 6 times the mean human C_{max} value and 22, 6, and 5 times the mean human AUC_{0-24} value in mice, rats, and monkeys, respectively. In reproductive and develop-

TABLE 4. Survival rates and potential causes of deaths identified by study pathologist in a 2-year carcinogenicity study in Sprague-Dawley rats

Survival parameter	Survival result by sex and dose (mg/kg/day)[a]							
	Male				Female			
	0	500	1,000	2,000	0	500	1,000	2,000
Survival rate at week 78	65%	52%	55%	52%	58%	58%	59%	58%
Survival rate at week 85	55%	42%	47%	38%	49%	45%	52%	38%
Survival rate at end of study[b]	38%	25%	25%	31%	34%	28%	34%	23%
Total unscheduled deaths	40	49	49	45	43	47	43	50
Undetermined	11	19	12	13	3	4	1	1
Gavage-related	2	1	3	1	0	0	0	0
Trauma/accidental	0	0	2	1	0	0	1	1
Pituitary adenoma	16	17	12	13	30	24	27	35
CPN	4	2	8	9	0	0	0	3
Granulocytic leukemia	0	2	0	0	0	0	0	0
Mammary carcinona	0	0	0	0	6	5	2	3
Mammary fibroadenoma	0	0	0	0	1	7	8	3
Urinary tract hemorrhage/necrosis/inflammation	1	3	3	1	0	0	0	0
Endometrial stromal sarcoma	0	0	0	0	0	0	0	2
Histiocytic sarcoma	0	0	2	0	0	2	0	0
Fibrosarcoma	0	1	2	0	0	1	0	0
All others[c]	6	4	5	7	3	4	4	2

[a] Animals were dosed for 95 weeks for groups 1, 2, and 3 and for 85 weeks for group 4.
[b] Animals were sacrificed at weeks 95 (group 2 males), 96 (groups 1, 3, and 4 males), and 97 (all females).
[c] The incidence of each individual cause was ≤1/sex/group.

mental toxicity studies, the steady-state animal-to-human exposure multiples at the NOAEL were 11 to 15 times the mean human C_{max} value and 6 to 14 times the mean human $AUC_{0\text{-}24}$ value in rats and were 20 times the mean human C_{max} value and 37 times the mean human $AUC_{0\text{-}24}$ value in rabbits.

DISCUSSION

Telbivudine had no safety pharmacology concerns in rats and monkeys and in the in vitro hERG assay. No acute toxicity was observed in rats or monkeys following oral administration of single doses up to 2,000 mg/kg/day.

In the repeat-dose studies, except for equivocal axonopathic findings in monkeys, occasional emesis, and minor changes in body weight and food consumption, no evidence of target organ toxicity was identified in mice, rats, or monkeys.

Telbivudine did not result in reproductive or developmental toxicity in rats or rabbits. Although slightly lower pregnancy rates were observed in the initial combined segment I and II study in rats, this finding was proven to not be attributable to the administration of telbivudine in the two subsequent confirmatory studies. The single abortion and two premature deliveries in rabbits dosed at 1,000 mg/kg/day were more likely due to maternal toxicity, i.e., reduced food consumption, reduced body weight, and abnormal feces. In fact, abortion and premature delivery are not uncommon in rabbits (7).

Telbivudine is nongenotoxic in a standard battery of genetic toxicity testing. The CB6F1-Tg *ras*H2 mouse has been developed as an alternate to the lifetime mouse bioassay to predict the carcinogenic potential of chemicals (13, 21, 28). The CB6F1-Tg *ras*H2 mouse is sensitive to both genotoxic and nongenotoxic carcinogens. The CB6F1-Tg *ras*H2 mouse model appears to be a better predictor for human carcinogenicity than the conventional mouse bioassay because it detects

TABLE 5. Incidence of selected tumors and CPN in a 2-year carcinogenicity study in Sprague-Dawley rats

Tumor type	Incidence by sex and dose (mg/kg/day)[a]							
	Male				Female			
	0	500	1,000	2,000	0	500	1,000	2,000
Pituitary adenoma	41/65	32/65	34/65	29/65	54/65	51/65	54/65	57/65
Adrenal pheochromocytoma	6/65	3/65	7/65	7/65	1/64	4/64	1/65	6/65
Pancreatic acinar cell adenoma	0/65	3/64	0/65	5/64	0/65	0/65	1/65	0/65
Mammary fibroadenoma	0/65	0/65	0/65	0/65	19/63	26/64	22/65	26/63
Mammary carcinoma	0/65	0/65	0/65	0/65	19/63	19/64	14/65	15/63
CPN	63/65	64/65	65/65	64/65	60/65	63/65	62/65	63/65
Mean severity[b]	2.6	2.5	3.0	2.8	1.3	1.3	1.4	2.0

[a] Animals were dosed for 95 weeks for groups 1, 2, and 3 and for 85 weeks for group 4.
[b] Grades for severity: 1, minimal, the least amount of change observed with the light microscope; 2, slight, less than average amount of change, but readily discernible as abnormal; 3, moderate, the average amount of change expected for a lesion; 4, marked, a marked amount of change with possible loss of function of the affected cells or organs; 5, severe, a great amount of change with probable loss of function of the affected cells or organs and frequently involving large areas of the organ.

TABLE 6. Steady-state exposure to telbivudine at the NOAEL and animal-to-human exposure multiples in mice, rats, rabbits, and monkeys

Study description	NOAEL (mg/kg/day)	C_{max}[a] [μg/ml (multiple)][b]	AUC_{0-24}[a] [μg · h/ml (multiple)][b]
13-wk toxicity in mice with toxicokinetics	3,000	141 (41)	595 (22)
6-mo toxicity in rats with 3-mo interim sacrifice	1,000	35.8 (11)	169 (6)
9-mo toxicity in monkeys with 3-mo interim sacrifice	500	21.1 (6)	126 (5)
Fertility, early embryonic development, and embryo-fetal development in male and female rats (segments I and II)	1,000	35.8 (11)	169 (6)
Fertility in male rats (segment I)	2,000	50.5 (15)	398 (14)
Fertility in female rats (segment I)	2,000	50.5 (15)	398 (14)
Embryo-fetal development in rabbits (segment II)	1,000	66.4 (20)	1,023 (37)
Multiple generation development study in rats (segment III)	1,000	35.8 (11)	169 (6)

[a] Animal steady-state C_{max} and AUC_{0-24} values were the averages of mean male and female data.
[b] Human steady-state C_{max} (3.4 μg/ml) and AUC_{0-24} (27.5 μg · h/ml) values were obtained from healthy subjects following 7 days of once daily oral administration of 600 mg of telbivudine (31), the approved therapeutic dose for patients with chronic HBV infection.

known or suspected human carcinogens at least as well as the conventional mouse bioassay and produces fewer positive results that are irrelevant to human carcinogenesis. These mice develop spontaneous and chemically induced neoplasms earlier in life and in greater numbers than wild-type mice. The most-common spontaneous neoplasms in control CB6F1-Tg *ras*H2 mice 8 to 9 months of age are lung adenoma and carcinoma (7.4% incidence), splenic hemangiomas and hemangiosarcomas (5.4%), forestomach squamous cell papillomas and carcinomas (2.4%), and skin neoplasms (1.2%) (21, 26).

Telbivudine was not carcinogenic at doses up to 2,000 mg/kg/day for 6 months in this transgenic mouse bioassay or in the 2-year conventional carcinogenicity study at doses up to 2,000 mg/kg/day in rats. In spite of the premature termination of this 2-year study, since at least 50% of survival rates were observed at week 78 in male and female animals of each group, carcinogenicity could be adequately assessed.

In the 2-year conventional carcinogenicity study, telbivudine appeared to be related to the death of a few rats due to advanced CPN. However, there was no evidence for an increased severity of nephropathy based on mean severity scores/group nor an increased overall incidence of nephropathy in any treated group.

CPN is a common, age-related renal disease affecting all conventional strains of rat used in safety evaluation studies, and in particular, the most commonly used Fischer 344 and Sprague-Dawley strains (10, 11, 20, 23). CPN occurs in both sexes of rat but at higher incidence and with progressively greater severity in males than in females, as seen in the 2-year carcinogenicity study. A number of factors, mainly dietary manipulations, have been shown to modify the expression of CPN. Among these, restriction of caloric intake is the most effective for inhibiting the disease process. As rats had access to feed ad libitum during the study, this may explain why the high incidence of this disease was observed. Many chemicals are known to exacerbate the severity of CPN to an advanced stage. However, the exacerbation of CPN by telbivudine can be regarded as having no relevance for extrapolation in human risk assessment because CPN is a rodent-specific disease (11).

In conclusion, comprehensive nonclinical safety studies were conducted at many multiples of the human exposure at 600 mg/day telbivudine. These studies demonstrated that telbivudine showed no evidence of significant adverse effects in both in vitro and in vivo studies that evaluated safety pharmacology, acute and chronic toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity, demonstrating a favorable safety profile of telbivudine.

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