CENTER FOR DRUG EVALUATION AND RESEARCH

21-797
21-798

APPLICATION NUMBER:

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-797**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09/30/04**
PRODUCT:	**Entecavir**
INTENDED CLINICAL POPULATION:	**Chronic Hepatitis B Virus infection**
SPONSOR:	**Bristol-Myers Squibb Company**
DOCUMENTS REVIEWED:	**Electronically**
REVIEW DIVISION:	**Division of Antiviral Drug Products (HFD-530)**
PHARM/TOX REVIEWER:	**Pritam S. Verma, Ph.D.**
PHARM/TOX SUPERVISOR:	**James G. Farrelly, Ph.D.**
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PROJECT MANAGER:	**Marsha Holloman**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

B. Recommendation for nonclinical studies: To support clinical use, the nonclinical toxicity profile of entecavir was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in rats and mice. The pivotal toxicology studies supporting the safety of entecavir were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of entecavir in humans.

C. Recommendations on labeling: The issue of labeling will be carried out separately.

II. Summary of nonclinical findings

A: Brief overview of nonclinical findings: Entecavir is an antiviral agent that is being developed for the treatment of chronic hepatitis B virus infection in humans. The recommended doses of entecavir are 0.5 mg (AUC = 14.97 ng*hr/ml) daily in nucleoside-naive patients and 1 mg (AUC = 26.55 ng*hr/ml) daily in lamivudine refractory patients.

The pharmacokinetic (PK) characteristics of entecavir in mice, rats, rabbits, dogs, and monkeys are comparable to those in humans indicating the acceptability of these species for the toxicological assessment of entecavir. The effective T-half in humans is approximately 24 hr. Serum protein binding of entecavir was low across animal species and humans (ranging from 8 to 24%), suggesting that there is little potential for drug interactions by displacement of other highly protein-bound drugs from their protein binding sites.

In both animals and humans, entecavir is almost entirely eliminated through the kidneys with parent drug as the major form present in urine. Only Phase II metabolites (glucuronides and sulfates) of entecavir were found in animals and humans and all of the metabolites identified in humans were present in the species used in the toxicological evaluation of entecavir. The total amount of metabolites, as a percentage of the total radioactive dose in excreta (urine and feces), was low in all species studied (eg, ~30% in animals and ~10% in humans). Entecavir is not a substrate, inhibitor, nor an inducer of the CYP isozymes and the PK of entecavir will neither effect nor be affected by the co-administration of agents that are either metabolized by, inhibit, or induce the CYP system.

Species-specific, reversible CNS inflammation was seen in dogs administered doses that achieve ~51 times the exposure to entecavir in humans at clinically proposed doses. The species specificity, reversibility, and high exposure multiples at which the CNS

inflammation was seen lead to the conclusion that this is probably not relevant to human safety. Other target organs in repeat-dose studies in animals were the kidneys, liver, lungs, skeletal muscle and testis; the changes in these organs were considered unlikely to be relevant to human safety because they were either species specific, associated with high exposure multiples relative to humans, and/or, in clinical trials with entecavir, they were not target tissues. The data from the 1-year study in monkeys indicated that there was no target organ toxicity in monkeys at exposures to entecavir ~136 times those in humans.

In a battery of genetic toxicology studies, entecavir was negative, with the exception of an in vitro chromosomal aberration test in primary human lymphocytes (without metabolic activation). These data indicate that entecavir is a genotoxic molecule. Carcinogenicity studies in CD-1 mice and SD rats were conducted. Increased incidence of tumors was observed in both the studies. The results of these studies were presented to the Executive Carcinogenicity Assessment Committee (ECAC) on June 17, 2003. The outcomes of the two studies were as follows:

Rat Carcinogenicity Study: The oncogenicity potential of entecavir was investigated in male rats at oral gavage dosages of 0.003 (low), 0.02 (mid), 0.2 (high) or 1.4 mg/kg/day (highest) and in females at dose levels of 0.01 (low), 0.06 (mid), 0.4 (high) or 2.6 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The Executive CAC committee found that the study was adequately designed and conducted.

The NOEL for neoplasia was 0.2 mg/kg/day for males and 0.06 mg/kg/day for females. At tumorigenic doses, systemic exposures were 35- and 4-times that in humans (1.0 mg daily dose) in male and female rats, respectively.

Treatment-Associated Tumors:

1. Hepatocellular adenomas in female rats were significant (p=0.005) at the highest dose level. Combined adenomas and carcinomas in the female rats were also significant (p=0.005) at the highest dose. In female rats, the combined incidence of adenomas and carcinomas was 1% (controls), 4% (low), 5% (mid), 2% (high) and 18% (highest).

2. Brain gliomas were significant (p=0.025) at the highest dose in both male and female rats. In male rats, the incidence was 0% (controls), 2% (low), 2% (mid), 3% (high) and 7% (highest). In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 0% (high) and 5% (highest).

3. The skin fibromas in female rats were significant (p=0.025) at the high and highest doses. In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 3% (high) and 5% (highest).

Mouse Carcinogenicity Study: The oncogenicity potential of entecavir was investigated in mice at oral gavage dosages of 0.004 (low), 0.04 (mid), 0.4 (high) or 4.0 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The Executive

CAC committee found that the study was adequately designed and conducted.

The NOEL for neoplasia was 0.004 mg/kg/day for males, based on pulmonary adenomas; for all other tumors in males and females, the NOEL was 0.4 mg/kg/day. At the tumorigenic dose in male mice, systemic exposure was 3-times that in humans (1.0 mg daily dose).

Treatment-Associated Tumors:

1. Lung adenomas were significant (p=0.005) in male mice (mid, high and highest) and in the female mice at the highest dose (p=0.005); lung carcinomas in both male and female mice were significant (p=0.005) at the highest dose. Combined lung adenomas and carcinomas were significant (p=0.005) in male mice at the mid, high and highest dose levels and in the female at the highest dose level (p=0.005). In male mice, the combined incidence of adenomas and carcinomas was 12% (controls), 20% (low), 26% (mid), 40% (high) and 58% (highest). In female mice, the combined incidence of adenomas and carcinomas was 20% (controls), 13% (low), 10% (mid), 35% (high) and 52% (highest).

2. Hepatocellular carcinomas in male mice were significant (p=0.005) at the highest dose level. In male mice, the combined incidence of adenomas and carcinomas was 11% (controls), 9% (low), 8% (mid), 16% (high) and 25% (highest).

3. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant (p=0.005) at the highest dose level. In female mice, the incidence of vascular tumors was 16% (controls), 23% (low), 29% (mid), 26% (high) and 64% (highest).

The ECAC found that the carcinogenicity studies in mice and rats were adequately designed and conducted. The committee judged the results of entecavir carcinogenicity studies. They concluded that entecavir was a carcinogen in rodents. The committee concluded that entecavir produced tumors in both species and both genders, and these results suggest a potential cancer hazard to patients.

At the request of the sponsor, the results of the carcinogenicity studies were presented to the full CAC (CAC), the committee that has been designated as the arbiter of disputes between sponsor and review divisions regarding the relevance of results in carcinogenicity studies. The CAC met on January 7, 2005 and voted that hepatocellular adenomas and carcinomas in female rats, skin fibromas in female rats and brain gliomas in both male and female rats were relevant. The committee also agreed that in the mouse carcinogenicity study, liver tumors in males and vascular tumors in females as well as lung tumors in both sexes were relevant to human safety evaluation.

In reproductive and developmental toxicity studies in rats, entecavir demonstrated no effects on reproductive function or fertility, and no adverse findings in a perinatal/postnatal study at exposures ~ 90 times that in humans at 1 mg daily. In the pivotal embryo-fetal development study in rats wherein doses of 2, 20, and

200 mg/kg were evaluated, both maternal and embryo-fetal toxicities were evident at >20 mg/kg (eg, decreased maternal body weights at >20 mg/kg, 1 maternal death at 200 mg/kg, increased resorptions at > 20 mg/kg, and malformations of the tail and vertebrae at 200 mg/kg). The exposures to entecavir in female rats at the no-effect and threshold doses for toxicity are 28 and 212 times that in humans at 1 mg daily, respectively. In the pivotal embryo-fetal development study in rabbits wherein doses of 1, 4, and 16 mg/kg were evaluated, no evidence of maternal toxicity was evident at any dose. There was an increased incidence of resorptions with associated decreases in live-litter sizes, developmental delays in ossification of the hyoid, and an increased incidence of 13th rib in dose related manner. Thus, entecavir was a selective developmental toxicant in rabbits. A NOAEL could not be identified for embryo-fetal development in rabbits.

B. Pharmacological activity: Entecavir is efficiently phosphorylated to entecavir-triphosphate (TP) by cellular nucleoside kinases. By competing directly with the natural deoxyguanosine triphosphate (dGTP), entecavir-TP potently inhibits each of the 3 distinct activities of the HBV viral polymerase: priming, reverse transcription of first-strand DNA synthesis, and the DNA-dependent DNA polymerase activity responsible for second-strand DNA synthesis.

C. Nonclinical issues relevant to clinical use: The Exec CAC concluded that entecavir produced tumors in rats and mice and both genders, and these results suggest a potential cancer hazard to patients. Entecavir can be classified as Pregnancy Category C. Entecavir should be used during pregnancy only if clearly needed.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

Entecavir (BMS-200475), a cyclopentyl guanosine analog, is an inhibitor of hepatitis B virus (HBV). The triphosphate form of entecavir blocks human HBV reverse transcriptase activity, inhibiting both the priming and elongation steps of the virus replication. Entecavir is being developed for the treatment and prevention of chronic HBV infection in adults.

The oral bioavailability of entecavir is approximately 37% to 80% in rats, 91% or greater in dogs and 16% in monkeys. Following intravenous administration to rats and dogs, entecavir has a steady state volume of distribution (1.02 to 4.63 L/kg) that is higher than the total body water, which suggests extravascular distribution and tissue protein biding. The human serum protein binding of entecavir is low (approximately 13%) indicating that there is a very low potential for drug-drug interaction due to displacement of protein bound drugs when coadministered with entecavir. Studies in pregnant and lactating rats indicate that entecavir penetrated the placenta and distributed into the fetus and that entecavir and/or its metabolite(s) were secreted into milk. The studies also indicated that entecavir crossed the blood/brain barrier in pregnant female rats and the blood/testis barrier in male rats. One glucuronide and 2 sulfate conjugates of entecavir have been identified in the urine of rats, dogs and monkeys in vivo but the contribution of these metabolites to the overall metabolite profile is relatively small, especially in dogs and monkeys. Entecavir is not an inhibitor of human cytochrome P450 isozymes 1A2, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4, suggesting that it does not have the potential to alter the human metabolic clearance of drugs that are metabolized by these major cytochrome P450 isozymes. The terminal elimination half-life after intravenous administration is 2.1 to 4 hr in rats, 3.8 to 9 hr in dogs and 6 hr in monkeys. A majority of the administered intravenous dose is recovered unchanged in the urine of rats (51% to 68%), dogs (72% to 76%) and monkeys (60%); after oral administration, the urinary recovery of the intact drug is 44% in rats, 63% to 83% in dogs and 12% in monkeys.

Entecavir has been evaluated in a number of toxicology studies including single dose oral studies in rodents, 2-week, 3-month and 6-month oral studies in rodents, 3-month studies in dogs, and 3-and 12-month studies in monkeys. Entecavir was also tested for oral teratology in rats and rabbits, oral studies of fertility and early embryonic development and pre- and postnatal development in rats and a battery of genetic toxicology studies. In addition, 2-year oral carcinogenicity studies and investigative studies with entecavir in mice and rats were performed. General toxicology studies in a variety of animals have shown that target organs for toxicity may include liver, bone marrow, lungs, kidneys, intestine, testes, and skeletal muscle. In a 6-month oral toxicology study of entecavir in mice at doses of 0.2, 1.0 or 5.0 mg/kg/day, liver was the target organ at all the doses. In addition, skeletal muscle and lung were the target organs at the mid or high doses. In a 6-month oral toxicology study of entecavir in rats at doses of 0.02, 0.08 or 0.3 mg/kg/day, degenerative changes in the liver were observed at all doses. A one year oral toxicity study in cynomolgus monkeys, revealed no significant toxicity other than minimal

elevation of BUN and potassium at exposures of 125 to 1000-fold greater than those predicted of human studies. In dogs at dose levels of 0.3, 3.0 or 30 mg/kg/day, adverse effects were evident at all doses and organs/tissues known to be targets included bone marrow, testes, kidneys, prostate glands, liver and inflammation in the CNS was observed at all doses. CNS lesions were not seen in dogs that were sacrificed in moribund condition after 1-month of dosing. CNS lesions were also not seen in rodents or monkeys. The CNS lesion was shown to be reversible and appeared to be specific to dogs of all the species studied.

Two-year oral carcinogenicity studies in rats showed significant incidence of hepatocellular adenomas and carcinomas in females, brain gliomas in males and females, and skin fibromas in females at the highest dose. In the two-year oral carcinogenicity studies in mice, combined incidence of lung adenomas and carcinomas was significant in males and females. Combined incidence of hepatocellular adenomas and carcinomas in males were significant at the highest dose level. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant at the highest dose level.

In an oral study of embryo-fetal development in rabbits, entecavir at dose levels of 1.0, 4.0 or 16.0 mg/kg caused embryo-fetal toxicity, delays in ossification of the hyoid and an increased incidence of 13th rib in a dose related manner. The exposures (AUCs) were 5.6 and 23.4μg*hr/ml at the mid and high dose, respectively. The exposures of rabbits to entecavir were 210 and 879 times, respectively, that in humans at the maximum therapeutic dose (1.0 mg, 26.6 ng*hr/ml). With regard to genotoxicity, entecavir was tested for its potential to induce chromosome aberrations in cultured human lymphocytes and it was found to be clastogenic.

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Information to sponsor: No

Sponsor and/or agent: Bristol-Myers Squibb Company
5 Research Parkway
Wallingford, CT 06492

Manufacturer for drug substance: Bristol-Myers Squibb Company
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Reviewer name: Pritam S. Verma, Ph.D.

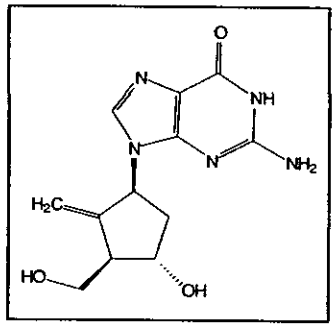
Division name: Division of Antiviral Drug Products

HFD #: 530

Review completion date: 11/03/04

Drug:

Trade name: BARACLUDE
Generic name: Entecavir
Code name: BMS-200475
Chemical name: 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one monohydrate
CAS registry number: 142217-69-4
Molecular formula: $C_{12}H_{15}N_5O_3.H_2O$
Molecular weight: 295.3
Structure:



Relevant IND: 52,196

Drug class: Nucleoside analog

Intended clinical population: Hepatitis B Virus (HBV) infected adults

Clinical formulation: Capsule dosage form

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-797 are owned by Bristol-Myers Squibb Company or are data for which Bristol-Myers Squibb Company has obtained a written right of reference. Any information or data necessary for approval of NDA 21-797 that Bristol-Myers Squibb Company does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Bristol-Myers Squibb Company does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-797.

Studies reviewed within this submission:

Table 1
Nonclinical toxicology studies

Study type/duration	Route of administration	Test system
Single dose	Oral	Rat and mouse
Repeat dose		
1. 2 week	Oral	Mouse, rat and dog
2. One month	Oral	monkey
3. 3 month	Oral	Mouse (diet), rat (diet) and dog
4. 6 month	Oral	Mouse and rat
5. One year (3-month interim)	Oral	monkey
Genetic toxicology		
6. Ames	In vitro	Salmonella and E. Coli
7. In vitro transformation	In vitro	Human lymphocytes
8. micronucleus	Oral	Rat
9. DNA repair	Oral	rat
Reproductive/developmental		
10. Fertility/embryonic development	Oral	Rat
11. Embryo-fetal development	Oral	Rat and rabbit
12. Pre- and post-natal development	Oral	Rat
Carcinogenicity	Oral	Rat and mouse
Safety pharmacology	In vitro	Ion channels, Purkinje fibers and enzyme systems

Table 2
Nonclinical pharmacokinetic studies

Type of study	Test system	Method of administration
2.6.5.3 Pharmacokinetic studies after a single dose		
Plasma concentration	Mice	Single, po
Mass balance	Rats	Single, po, iv
Plasma concentration and bioavailability	Rats	Single, po,iv
Plasma concentration	Woodchucks	Single, po
Plasma concentration, bioavailability and urinary excretion	Dogs	Single, po, iv
Mass balance	Dogs	Single, po, iv
Mass balance	Monkeys	Single , po, iv
2.6.5.4 Pharmacokinetic after repeated doses		
Plasma concentrations	Mice	Multiple, po
Plasma concentrations	Rats	Multiple, po
Plasma concentrations	Rabbits	Multiple, po
Plasma concentrations	Dogs	Multiple, po
Plasma concentrations	Monkeys	Multiple, po
2.6.5.5 Organ distribution		

Tissue distribution of radioactivity	Mice	Single, po
Tissue distribution of radioactivity	Rats	Single, po
2.6.5.6 Protein binding and distribution		
In vitro serum protein binding	Mice, rats, dogs, monkeys, humans	N/A
2.6.5.8 Metabolism in vivo		
Comparative in vivo metabolism	Rats, dogs and monkeys	Single, po, iv
Biotransformation profiles in plasma, urine, feces, liver and lung	Mouse, rats	Single, po

Studies not reviewed within this submission: exploratory studies in mice and rats were not reviewed.

2.6.2 PHARMACOLOGY

Mechanism of action

Entecavir is efficiently phosphorylated to ETV-TP by cellular nucleoside kinases. By competing directly with the natural deoxyguanosine triphosphate (dGTP), ETV-TP potently inhibits each of the 3 distinct activities of the HBV viral polymerase: priming, reverse transcription of first-strand DNA synthesis, and the DNA-dependent DNA polymerase activity responsible for second-strand DNA synthesis. In addition to competing directly with dGTP, ETV-TP was a non-obligate chain terminator of HBV DNA synthesis. Entecavir is a selective and potent inhibitor of HBV (ie, the effective concentration for inhibition of 50% of virus yield [EC_{50}] = 0.00375 μM).

2.6.2.1 Brief summary

1. Entecavir: effects of on rabbit Purkinje fiber action potentials: In an in vitro experiment, entecavir was tested at doses of 0 (vehicle control), 3, 10 or 30 μM for effects on rabbit-Purkinje fiber action potentials using conventional intracellular techniques. Entecavir had no significant effect on any of the electrophysiological parameters of Purkinje fiber action potential. Conclusions: entecavir was negative in producing cardiac electrophysiological effects under the conditions of the experiment.

2. Entecavir: Effect on cloned hERG channels expressed in mammalian cells (I): The effect of entecavir on hERG current was measured at concentrations of 0 (vehicle control), 3, 10 and 30 μM on the hERG current (IKr, the rapidly activating, delayed rectifier cardiac potassium current). Entecavir minimally inhibited hERG current but the inhibition was not biologically significant. These results were comparable to the vehicle controls. The positive control, terfenadine at 60 nM blocked 86.8% of hERG current. In conclusion: entecavir did not produce biologically meaningful inhibition of hERG current.

3. Entecavir: Effect on L-type calcium channels and canine Purkinje fiber action potentials: The effect of entecavir on cardiac L-type calcium channel currents in canine ventricular myocytes and canine Purkinje fiber action potentials was measured at concentrations of 0 (vehicle control), 3, 10 and 30 μM. Entecavir had no significant effect on any of the action potential parameters tested. In conclusion: entecavir did not produce a biologically significant effect on L-type calcium channel currents measured in canine ventricular myocytes and on action potentials in canine Purkinje fibers. The canine Purkinje fiber results are consistent with results obtained using rabbit Purkinje fibers in a previous study.

2.6.2.4 Safety pharmacology

Cardiovascular effects:

1. **Entecavir: effects of on rabbit Purkinje fiber action potentials, March 22, 2001 (920010881)**

In an in vitro experiment, entecavir was tested at doses of 0 (vehicle control), 3, 10 or 30 μM for effects on rabbit-Purkinje fiber action potentials using conventional intracellular techniques. Running Purkinje fibers with ventricular muscle attached at both extremities were carefully excised from the left ventricles of New Zealand White rabbit hearts. Action potential parameters measured included resting membrane potential, overshoot, maximal upstroke velocity and time to 50% and 90% repolarization. Results: entecavir had no significant effect on any of the electrophysiological parameters of Purkinje fiber action potential. Conclusions: entecavir was negative in producing cardiac electrophysiological effects under the conditions of the experiment.

2. **Entecavir: Effect on cloned hERG channels expressed in mammalian cells (I), November 11, 2003 (030515.CNT/DN3051/DN03110)**

The effect of entecavir on hERG current was measured at concentrations of 0 (vehicle control), 3, 10 and 30 μM on the hERG current (IKr, the rapidly activating, delayed rectifier cardiac potassium current). HEK293 cells were maintained in cryogenic storage. The cells used for electrophysiology were plated in plastic culture dishes. Results: mean percentage of current inhibited at each entecavir concentration is shown in Table 1. Entecavir minimally inhibited hERG current but the inhibition was not biologically significant. These results were comparable to the vehicle controls. The positive control, terfenadine at 60 nM blocked 86.8% of hERG current. In conclusion: entecavir did not produce biologically meaningful inhibition of hERG current.

Table 1
Mean percentage of current inhibited at each entecavir concentration

Test article	Concentrations (μM)	N	% mean inhibition
Entecavir	0	3	0.5
	3	3	0.2
	10	3	0.8
	30	3	0.9
terfenadine	60 nM	2	86.8

3. Entecavir: Effect on L-type calcium channels and canine Purkinje fiber action potentials, June 24, 2004, (930007381)

The effect of entecavir on cardiac L-type calcium channel currents in canine ventricular myocytes and canine Purkinje fiber action potentials was measured at concentrations of 0 (vehicle control), 3, 10 and 30 μM. Conventional intracellular and whole cell patch clamp techniques were used to determine the effects of entecavir on canine Purkinje fiber action potential and on canine ventricular L-type calcium currents. Action potential parameters measured included resting membrane potential, overshoot, maximal upstroke velocity (Vmax) and time to 50% (APD_{50}) and 90% (APD_{90}) repolarizations. Results: in canine cardiac Purkinje fiber assay, entecavir had no significant effect on any of the action potential parameters tested (Table 2). These results were comparable to the vehicle controls. The positive control, nisoldipine inhibited L-type calcium currents by 41.2%, 78.7% and 95.5% at 3, 10 and 30 nanomolar concentrations, respectively. In conclusion: entecavir did not produce biologically significant effect on L-type calcium channel currents measured in canine ventricular myocytes and on action potentials in canine Purkinje fibers. The canine Purkinje fiber results are consistent with results obtained using rabbit Purkinje fibers in a previous study.

Table 2
Effect of entecavir on canine Purkinje fiber action potentials (n=4)

Parameters	Vehicle control (0)	Concentrations (μM)		
		3	10	30
Vmax	590.6	584.4	576.8	560.1
APD_{50}	228.6	226.9	225.1	224.5
APD_{90}	295.8	293.8	294.6	294.1

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Absorption, Bioavailability, and Pharmacokinetics

Entecavir was rapidly absorbed following oral administration in mice, rats, woodchucks, rabbits, dogs, and ducks. In monkeys, the absorption rate was slower and the extent of absorption was lower relative to other species (eg, Tmax in monkeys was 2 hours Compared to ~1.5 hours in rats and dogs and the extent of absorption was approximately 16% compared to > 80% in rats and dogs).

The apparent terminal plasma half-life (T-half) of entecavir following intravenous (IV) administration ranged from approximately 2 to 9 hours in selected species (rats, dogs, and monkeys). In contrast, the mean T-half of entecavir was approximately 130 hours in humans. The difference between theT-half values in animals and humans is related to the longer sampling scheme and more sensitive assays used in human studies, which allowed for the detection of low concentrations of entecavir over a prolonged period of time. The prolonged T-half observed in humans probably reflects the return of entecavir from intracellular sites, as the area under the terminal phase (starting at 48 hours post dose) represents approximately 20% of the total area under the concentration vs time curve (AUC). This also explains why the steady-state accumulation following once daily administration ranged from 1.6 to 2.7 at doses ranging from 0.1 to 1 mg/day. There was no indication of systemic accumulation of entecavir after repeated administration in rats and monkeys, although, similar to humans, there was low to moderate accumulation in dogs (1.1- to 2.5-fold increase). Values for the systemic clearance (CLT) of entecavir in rats, dogs, and monkeys ranged from 8.6 to 46.7 mL/min/kg. In contrast, the estimated CLT value for entecavir in humans (approximately 6-7 mL/min/kg) was lower than in animals based on an estimated bioavailability of 70 to 80% and an extended mean T-half compared to animals.

Distribution

Values for the steady-state volume of distribution (VSS) in rats (4.6 L/kg), dogs (1.6 L/kg), and monkeys (1 L/kg) were greater than the volume of total body water in these species (approximately 0.7 L/kg), suggesting extravascular distribution of the drug and/or preferential binding in tissues. Similarly, the apparent volume of distribution of entecavir after an oral dose in humans was estimated to be large (ie, this value in humans could only be estimated as data following intravenous dosing are not available). Tissue distribution studies in mice and rats indicated that entecavir was rapidly and extensively distributed throughout the body with no sex differences in tissue distribution. The highest percentages of dosed radioactivity were associated with GI-tract tissues, liver, and kidney (liver and kidney were target tissues in 6-month rodent toxicology studies). This is not surprising since entecavir was dosed orally and was mainly eliminated in the urine. The distribution of radioactivity to the liver indicates that entecavir is able to reach its intended site of action. Other target tissues in rodent toxicology/carcinogenicity studies

(eg, brain, lung, skeletal muscle, and testis) also showed evidence of exposure to entecavir. The elimination of radioactivity occurred rapidly, with most of the administered radioactivity recovered in 24 hours. Drug-related radioactivity was widely distributed in maternal and fetal tissues of pregnant rats, and was secreted in the milk of lactating rats within 1 hour after oral administration of [14C]-entecavir. These findings indicate the potential for fetal and neonatal exposure to entecavir and/or its metabolites if the drug is administered to pregnant or lactating women. In addition, [14C]-entecavir derived radioactivity was detected in maternal cerebrum in pregnant rats, and dose-related increases in exposure to entecavir were found in the CSF of mice, rats, dogs, and monkeys in other toxicokinetic studies. Collectively, these data indicate that entecavir can cross the blood/brain barrier.

Metabolism

Because entecavir is eliminated mainly as unchanged drug in the urine, biliary clearance plays a minor role. The majority of the drug-related components in feces were unchanged entecavir. Only Phase II metabolites of entecavir were found in rats, dogs, monkeys, and humans. All of the metabolites identified in humans were present in the species used in the toxicological evaluation of entecavir. In general, the animal species were exposed to higher amounts of the Phase II metabolites than were humans, and the types of Phase II metabolites identified in the animal species are generally considered to be less toxic than the parent compound. Two sulfate conjugates (M2 and M3) and 3 glucuronide conjugates (M1, M4, and M5) of entecavir were detected in male and female rats and male monkeys; in dogs, the glucuronides M1, M4, and M5. In humans, 4 Phase II metabolites of entecavir were detected, namely, M1, M2, M4, and M5 of entecavir were detected. The total amount of metabolites, as a percentage of the total radioactive dose in excreta (urine and feces), was low in all species studied (eg, 30% in animals and 10% in humans). In rats, the total amount of metabolites as a percentage of radioactivity in excreta was approximately 10% or less in male rats and approximately 30% in female rats. The metabolite M2 was the major metabolite in female rats, accounting for the main difference in the extent of metabolism in male and female rats (ie, metabolites constituted a higher percentage of the total radioactivity in plasma, urine, and feces in female rats). Additional in vitro findings indicate that entecavir is not a substrate, inhibitor, or inducer of the CYP isozymes. These results are consistent with the observation that entecavir is not subject to oxidative metabolism and indicate that entecavir has little potential to inhibit the metabolic clearance of drugs that are highly metabolized by the major human CYP isozymes. Furthermore, the pharmacokinetics of entecavir in humans is unlikely to be affected by the co-administration of agents that are either metabolized by, inhibit, or induce the CYP system. Likewise, the pharmacokinetics of known CYP substrates is unlikely to be affected by the co-administration of entecavir.

In vitro biotransformation studies indicated that there was no significant metabolism of entecavir in mouse, rat, dog, monkey, and human liver microsomes or S9 fractions; dog and monkey hepatocytes; and dog, monkey, and human liver slices. Only rat hepatocytes and rat liver slices metabolized entecavir to an appreciable extent, and metabolite M1 was the only metabolite identified. The biotransformation of [14C]-entecavir was also

investigated in mouse lung microsomes, mouse and rat lung homogenates, and mouse and rat lung S9 fractions. No oxidative metabolism was detected in any of these in vitro studies.

Entecavir, at concentrations up to 300 μM (approximately 83μg/ml which is approximately 10,000 times higher than the steady-state Cmax in humans of 8.3 ng/mL at 1 mg), was not an inhibitor of the major human cytochrome P450 (CYP) isoforms (1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4) involved in drug metabolism. In addition, entecavir, at concentrations up to 10 μM (approximately 2.8 μg/mL, which is approximately 340 times higher than the steady-state Cmax in humans at 1 mg), did not induce the human CYP isoforms 1A2, 2B6, 2C9, 2C19, and 3A4/5.

Excretion

Mass balance studies in rats, dogs, and monkeys indicated that [14C]-entecavir was eliminated in the urine and feces. In rats and dogs, after either IV or oral administration, the excretion of radioactivity (as a % of dose) ranged from approximately 63 to 84% and 1.4 to 33% in the urine and feces, respectively; the overall recovery ranged from 88 to 97%. In monkeys, the excretion of radioactivity was 77% and 5.5% in the urine and feces, respectively, after an IV dose, and it was 13% and 78% in the urine and feces, respectively, after an oral dose. Overall recovery of radioactivity following IV and oral doses was 90% and 95%, respectively. In these animal studies, unchanged entecavir accounted for the majority of radioactivity recovered in the urine. Following a single oral 1-mg dose of [14C]-entecavir in humans, the mean urinary recovery of entecavir and radioactivity over 336 hours accounted for approximately 70% and 76% of the dose, respectively, and fecal recovery accounted for approximately 6%. Thus, of the total radioactivity excreted in urine, approximately 90% was entecavir. In rats, monkeys, and humans, renal clearance values are greater than the glomerular filtration rate (GFR) values, suggesting that entecavir is eliminated through a combination of passive (glomerular filtration) and active (net tubular secretion) processes by the kidney.

Protein Binding

In vitro, the serum protein binding of entecavir was low in mice, rats, dogs, monkeys, and humans (ranging from 7.9 to 24%), and entecavir was uniformly distributed between plasma and red blood cells (RBC) in human blood. The low protein binding in humans (13%) suggests that there is little potential for drug interactions due to displacement of highly protein bound drugs when co-administered with entecavir. Protein binding was independent of serum drug concentrations within the entecavir concentration range of 50 to 5000 ng/mL.

Toxicokinetics

Dose-related systemic exposures to entecavir were established after multiple doses in the species used for nonclinical toxicology testing. Mean steady-state exposures in

toxicology studies 3 months in duration were as high as approximately 2500 times greater than the mean steady state exposure in humans at 1 mg. As previously mentioned, exposure to entecavir was generally higher in male rats than in females. The biotransformation results in rats indicated that this difference was attributable to the greater production of the major metabolite (M2) in female. In contrast to rats, there were no apparent sex-related differences in systemic exposures to entecavir in mice, dogs, monkeys, or humans. As previously described, entecavir was also detected in the CSF in mice, rats, dogs, and monkeys, indicating that entecavir can cross the blood/brain barrier in these species (CSF:plasma ratios ranged from approximately 0.2 to 0.6 in mice and rats, 0.03 to 0.06 in dogs, and 0.04 to 0.1 in monkeys.

2.6.4.2 Methods of Analysis

HPLC with fluorescence (FL) or MS/MS detection methods were used to determine concentrations of entecavir in selected matrices.

Plasma samples from mouse, rat, rabbit, and dog were analyzed initially with validated HPLC/FL detection assays, and in later studies, plasma or urine samples from mouse, rat, dog, and cynomologus monkey were analyzed for entecavir using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods. Due to the low extent of metabolism and the resulting low concentration of metabolites in plasma, only entecavir was analyzed in these studies. The procedures were sensitive and specific, using . The structural analogue, lobucavir (BMS-180194), was used as an internal standard throughout these studies.

The initial HPLC/FL method was used to measure entecavir in rat and dog plasma. Samples were prepared using and FL detection. No interfering substances with a retention time similar to those of entecavir or the internal standard were present in control plasma. This method was later cross-validated for the determination of entecavir in mouse plasma and rabbit plasma. Later methods for various biological matrices were all based on MS/MS instead of FL, because of the selectivity and higher sensitivity afforded by the mass-selective technique.

The use of different assays within a particular species for analysis of samples from studies did not have an impact on the validity of the derived PK data or its interpretation, since the assays were validated and the acceptance of the analytical results from an individual study was contingent upon the respective pre-established criteria being met. In no instance were two different methods used to analyze samples from a single study for a given analyte in a given matrix and a given species. However, due to the lack of commercially available control matrices to support sample analyses for woodchuck and duck studies, study samples from these studies were analyzed with a method that was fully validated in a surrogate matrix rather than in the corresponding matrix. In the woodchuck study (DCN 910064656), the samples were analyzed using a human plasma assay due to lack of commercially available woodchuck plasma. To ensure the validity of the assay, quality control (QC) samples prepared in woodchuck plasma were included

in each run, and the same acceptance criteria were applied to these QCs as for the human plasma QCs to ensure the validity of the assay. Similarly, for the study in ducks (DCN 920006931), a validated rat plasma assay was used for the sample analysis. To minimize the effect of matrix between rat and duck plasma, QC samples prepared in a duck/rat plasma mixture (1:3) were also included in each run to qualify the assay. All study samples were diluted with rat plasma in the same fashion as the QC samples to ensure the validity of the data.

The concentrations of radioactivity in plasma, milk, amniotic fluid, and urine samples, from studies in which radiolabeled entecavir was administered, were determined by direct liquid scintillation counting (LSC) of the samples mixed with scintillation cocktail. Blood samples, fecal homogenates, and various tissues/organs were either solubilized (eg, dissolved in sodium hydroxide (NaOH) solution) or combusted prior to radioactivity analysis by LSC.

2.6.4.3 Absorption

1. Summary report: Single dose iv and oral pharmacokinetics of entecavir in rats, July 19, 1995 (Report No. 910050874).

The single dose pharmacokinetics of entecavir after oral (25 mg/kg) and iv (10 mg/kg) administrations was investigated in male rats (n=3). Serial blood samples were obtained at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after the dosing and were analyzed by a validated HPLC method. Results: are shown in Table 3. After the oral administration, peak plasma concentrations occurred within 1.5 hr. By comparing the dose normalized AUCs after oral administration to the mean AUCs after iv administration, the oral bioavailability was estimated to be 37%.

Table 3

Single dose pharmacokinetics of entecavir in male rats after iv or po administration.

Dose (mg/kg)	Route	Cmax (µg/ml)	AUC (µg*hr/ml)	T½ (hr)	Vdss (l/kg)	Cl (l/kg*hr)	F (%)
10	IV	7.81	5.92	4	3.76	28.1	-
25	PO	1.22	5.47	5.2	-	-	37

2. Summary report: Pharmacokinetics of entecavir in woodchucks following oral administration (Report No. 910064656).

The single dose pharmacokinetics of entecavir after oral gavage (0.5 mg/kg) administration was investigated in two female woodchucks (age: 2 yrs). Serial blood samples were obtained at 0, 0.5, 1, 2, 6, 12, 24, 36, 48, 60 and 72 hr after the dosing and were analyzed by a validated HPLC method. Results: are shown in Table 4. Entecavir was absorbed when administered orally to woodchucks with peak plasma levels

occurring at 1 to 2 hr postdose.

Table 4
Single dose pharmacokinetics of entecavir in the female woodchuck after po administration (0.5 mg/kg).

Woodchuck No.	Tmax (hr)	Cmax (ng/ml)	AUC (ng*hr/ml)	T1/2 (hr)
1	1	—	—	—
2	2	—	—	—

3. Summary Report: Single dose pharmacokinetics of entecavir in dogs (Report No. 910059456).

The pharmacokinetics of entecavir was investigated in 3 male dogs after single iv (5 and 10 mg/kg) or oral (10 and 25 mg/kg) doses in a cross over study; the washout period was one week between the doses. Serial blood samples were taken at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after dosing and urine samples were taken up to 24 hr following the administration of each dose. The separated plasma and urine samples were analyzed by a validated LC/MS/MS method for entecavir concentrations. Results: are shown in Table 5. After either route of administration, over 60% of the dose was recovered as unchanged drug in the urine in 24 hr. Plasma concentration of the unchanged compound after the iv or po administrations was found to increase more than dose proportionally with respect to AUC and Cmax. The oral bioavailability was estimated to be 91% following the oral dose and urinary recovery of entecavir was 83%.

Table 5
Single dose pharmacokinetics of entecavir in male dogs after iv or po administration.

Dose (mg/kg)	Route	Cmax (µg/ml)	AUC (µg*hr/ml)	T½ (hr)	Vdss (l/kg)	Cl (l/kg*hr)	F (%)
10	IV	20.6	20.78	3.76	1.32	8.43	100
5	IV	7.4	7.25	9.31	1.62	11.75	100
10	PO	8.1	18.1	5.47	-	-	91
25	PO	26.5	71.3	3.3	-	-	-

4. Pharmacokinetics of entecavir in ducks following oral administration (Report No. 920006931).

The single dose pharmacokinetics of entecavir after oral gavage (0.5 mg/kg)

administration was investigated in two male Pekin Ayklesbury ducks (age: 7 months). Serial blood samples were obtained at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after the dosing and were analyzed by a validated LC/MS/MS method. Results: are shown in Table 6. Entecavir was absorbed when administered orally to ducks with peak plasma levels occurring at 0.5 hr postdose.

Table 6
Single dose pharmacokinetics of entecavir in male ducks after po administration (0.5 mg/kg).

Duck No.	Tmax (hr)	Cmax (ng/ml)	AUC (ng*hr/ml)	T2 (hr)
1	0.5	—	—	—
2	0.5	—	—	—

2.6.4.4 Distribution

1. Summary report: Protein binding of Entecavir to human plasma, 1/25/1996 (Report No. 910051558)

Protein binding of entecavir (10 μg/ml) in human plasma was measured by the ultrafiltration method at 37°C. Results: the mean binding to human plasma protein was determined to be 10.2%, and would not be expected to be clinically relevant.

2. Entecavir: Tissue distribution of radioactivity in mice following a single oral administration of ^{14}C-entecavir (study # 920001980)

Male and female CD-1 mice (21/sex) received a single oral gavage dose of ^{14}C-entecavir (10 mg/kg) to investigate a limited tissue distribution of radioactivity in mice. Blood and selected tissues were collected from 3 mice/sex at predose and 0.5, 4, 24, 48, 96 and 168 hr postdose and total radioactivity was determined by liquid scintillation counting. Results: the distribution of radioactivity was comparable in males and in females. Maximum levels of radioactivity occurred at 0.5 hr postdose for all tissues except large intestine (4 hr) and declined rapidly in majority of the tissues by 24 hr postdose, the concentrations in the tissue were generally <12% of the respective Cmax values excerpt for the brain (30 to 35%). Relative to the blood, low levels of radioactivity were detected at 0.5 hr postdose in the brain, eyes, large intestine, ovaries and testes; on the other hand, radioactivity was 1.1- to 49.5-fold higher in bladder, bone, carcass, heart, kidney, liver, lungs, muscle, skin, small intestine, spleen and stomach than the blood. The highest percentages of radioactivity were observed in the gastrointestinal tract tissues (small intestine, stomach) and kidneys. At 24 hr postdose, there was <0.5% of the radioactivity detected in the tissues. Conclusions: maximum levels of radioactivity occurred at 0.5 hr post dose and declined rapidly in majority of the tissues. The results indicated extensive distribution of entecavir and/or metabolites in mice following oral administration of ^{14}C-entecavir, with no apparent gender differences and <2% of the radioactivity remained in

the tissue and carcass at 24 hr after dosing. Almost complete recovery of the administered radioactivity was observed in 24 hr postdose.

3. Entecavir: Lacteal excretion and fetal tissue distribution of radioactivity in pregnant female Sprague-Dawley rats and tissue distribution of radioactivity in male and non-pregnant female rats following oral administration of ^{14}C-entecavir, 2/6/2003' — 6108-367)

Distribution of ^{14}C-entecavir derived radioactivity in maternal and fetal tissues, and lacteal excretion of radioactivity were assessed following a single oral administration of ^{14}C-entecavir to timed-pregnant and non-pregnant female and male rats. Sixty-nine rats were divided into six groups. Group 1 (n=2) and group 2 (n=1) were the control groups from which blood, milk and tissues were collected for matrix background substraction. Group 3 (n=21) consisted of pregnant females (18 days of gestation) from which blood and selected maternal and fetal tissues were collected at 1, 4, 8, 12, 24, 48 and 72 hr following an oral dose of 10 mg/kg (100 µCi/kg) of ^{14}C-entecavir. Group 4 (n=8) consisted of pregnant females (18 days of gestation) from which one animal/timepoint was sacrificed at predose, 1, 4, 8, 12, 24, 48 and 72 hr following an oral dose of 10 mg/kg (140 µCi/kg) of entecavir to examine tissue distribution of radioactivity by whole-body autoradiography (WBA). Group 5 (n=21)consisted of lactating females (7 days postpartum) from which blood and milk were collected at 1, 4, 8, 12, 24, 48 and 72 hr following an oral dose of 10/kg (100 µCi/kg) of entecavir. Group 6 (n=16, 8/sex) consisted of non-pregnant females and males from which one animal/sex/timepoint was sacrificed at predose, 1, 4, 8, 12, 24, 96 and 168 hr following an oral dose of 10 mg/kg (140 µCi/kg) of entecavir to examine tissue distribution of radioactivity by whole-body autoradiography. Results: pharmacokinetic parameters for tissues, blood, plasma and milk for animals in group 3 and 5 are shown in Table 7.

Table 7

Pharmacokinetic parameters for tissues, blood, plasma and milk for animals in group 3 and 5

Matrix (Group 3)	Cmax (µg equiv./g	Tmax (hr)	T-half (hr)	AUC (inf) (µg equiv*hr/g)
Amniotic fluid	0.134	1	16.7	1.3
Blood	2.0	1	15.4	7.6
Cerebrum	0.219	1	37.4	10.4
Heart	3.2	1	28.3	43.7
Kidneys	12.2	1	30.7	113
Liver	27.1	1	90.6	207
Lungs	8.0	1	34.8	48.1
Ovaries	5.0	1	31.5	42.8
Placenta	3.7	1	26.2	33
Plasma	2.4	1	40.6	9.7
Uterus	5.4	1	30.3	67.8
Fetal blood	0.51	1	13.5	4.7
Fetal brain	0.31	4	25.4	8.2
Fetal kidney	0.486	1	22.3	4.4
Fetal liver	0.81	1	18.8	9
Fetal carcass	0.499	1	16.8	5.2
Group 5				
Blood	2.8	1	19	9.3

Milk	0.55	1	15.1	3
Plasma	3.3	1	22.2	11.6

^{14}C-Entecavir derived radioactivity was widely distributed in both maternal and fetal tissues following a single oral administration. Radioactivity was detected in all collected maternal matrices through 72 hr postdose. In fetal tissues, radioactivity was detected in all matrices except fetal kidney at 48 hr postdose.

The mean maximum concentrations of radioactivity in maternal blood and plasma and fetal blood following a single oral administration of radioactive entecavir were 2.02, 2.4 and 0.519 μg equiv/g, respectively, at one hr postdose. Fetal tissue concentrations were highest in the liver, blood, carcass, kidneys and brain and lowest in the amniotic fluid.

Radioactivity in tissues reached mean maximum concentration levels (Tmax, Table 7) for all collected tissues at 1 hr post dose. Radioactivity was eliminated from the maternal blood and plasma with half lives of 15.4 and 40.6 hr, respectively. Radioactivity was eliminated from the fetal blood with half life values of 13.5 hr. Half life values in collected fetal tissues range from 6.8 hr (fetal carcass) to 25.4 hr (fetal brain).

The AUC values for maternal blood and plasma, and fetal blood were 7.6, 9.7 and 4.7 μg equiv/g respectively.

The mean maternal tissue: maternal plasma concentration ratios were greater than 1 for maternal tissues at all samples time points except blood and amniotic fluid.

Autoradiographic data for pregnant females were consistent with the results of excision experiments and indicated that low levels of drug-derived radioactivity crossed the placenta and blood/brain barrier. Quantifiable levels of radioactivity were detectable in fetuses. The maternal tissues with the highest concentrations of drug-derived radioactivity at 1 hr postdose (Cmax) were small intestinal contents, stomach contents, small intestine, liver and spleen.

Autoradiographic data from non-pregnant females and males showed high concentration in the GI tissues and contents, urine, spleen and liver.

Comments: overall, the results of this study indicated that ^{14}C-entecavir derived radioactivity penetrated the placenta and distributed into the fetus following a single oral dose of entecavir to pregnant rats, with generally lower levels of radioactivity in the fetal tissues than in the maternal tissues and that entecavir and/or its metabolites were secreted into milk following a single oral dose of entecavir to lactating rats. The results indicated that entecavir crossed the blood/brain barrier in pregnant female rats and the blood/testis barrier in male rats

4. Tissue distribution of radioactivity in rats following a single oral administration of ^{14}C-entecavir, 1/27/1999 , — , study no. 6108-304)

The distribution of radioactivity following a single oral administration was compared in males and females Sprague-Dawley rats. A single 10 mg/kg oral gavage dose of ^{14}C-entecavir was administered to 42 rats (21/sex). Blood and selected tissue were collected from 3 rats/sex at 1, 4, 8, 24, 48, 96 and 168 postdose and total radioactivity was determined by liquid scintillation counting. The results are shown in Table 8.

Table 8

Mean concentrations of entecavir in plasma and tissues 1 hr postdose following a single oral dose of ^{14}C-entecavir to rats

Tissue	Cmax (μg equiv/g)	Tmax (hr)	Half life (hr)	AUCinf ((μg equiv*hr/g)	AUC ratio
Adrenal glands	3.9	1	18	31.2	2.92
Bladder urinary	36.8	1	116	146.7	13.7
Blood	2.5	1	4	8.1	0.76
Bone (femur)	2.7	1	60	20.7	1.94
Bone marrow (femur)	15.7	1	8	44.52	4.16
Brain	0.24	1	87	21.0	1.96
Cerebrospinal fluid	0.095	1	-	24.3	2.28
Eyes	0.73	1	101	11.3	1.06
Fat	1.5	1	42	9.6	0.90
Heart	4.15	1	33	41.3	3.86
Kidneys	16.8	1	59	124.7	11.65
Large intestine	12.7	4	78	196.2	18.32
liver	20.1	1	150	194.7	18.19
Lungs	6.2	1	74	50.5	4.72
Lymph node, mesenteric	13.3	1	103	70.9	6.63
Muscle, thigh	2.5	1	168	95.8	8.01
Ovaries	3.6	1	55	30.6	2.86
Pancreas	3.9	1	85	59.3	5.54
Pituitary	2.6	1	11	21.2	1.99
Plasma	3.2	1	18	10.7	-
Prostate	7.5	1	38	76.9	7.18
Salivary glands	4.8	1	101	85.1	7.96
Skin	3.1	1	115	34.6	3.23
Small intestine	63.6	1	64	222.2	20.75
Spleen	23.7	1	40	95.3	8.9
Stomach	6.5	1	78	55.1	5.15
Testes	0.99	1	67	24.5	2.3
Thymus	10	1	47	82.6	7.71
Thyroid	3.0	1	15	26.4	2.47
Uterus	6.0	1	63	39.4	3.68

Maximum levels of radioactivity occurred at 1 hr postdose for all tissues except large intestine (4 hr) and declined rapidly in the majority of the tissues, by 24 hr postdose. The concentrations in the tissues were generally <10% of the respective Cmax values except for the brain (53%) and testes (20%). Relative to plasma, low levels of radioactivity were detected at 1 hr post dose in the cerebrospinal fluid, brain, eyes, testes, muscle, bone, pituitary and ovaries. The radioactivity was 1.3 to 7.7 folds higher in tissues, such as the pancreas, adrenal glands, uterus, prostate, thymus, lungs, bone marrow, lymph nodes, kidneys, liver and spleen than the plasma. The highest percentages of dose radioactivity were observed in the GI tract tissue, GI tract contents, carcass and liver. Half life values of radioactivity in the tissues varied from 4 to 168 hr. However, at 24 hr postdose, there were only 1.74% and 2.06% of the dosed radioactivity detected in the tissues and carcass, respectively. By 168 hr postdose, there were low or undetectable levels of radioactivity,

with less than 0.03% and 1.1% of the administered dose remaining the tissues and carcass, respectively. It was noted that these half life values were determined based on a very low level of radioactivity in the tissues.

Comments: overall, the results indicated extensive distribution of the drug and/or metabolites in rats following oral administration of ^{14}C-entecavir and almost complete recovery of the administered radioactivity in 24 hr. The distribution of radioactivity was rapid. Maximum levels of radioactivity occurred at 1 hr postdose for all tissues except the large intestine (4hr). The highest percentages of dosed radioactivity were observed in the GI tract tissues and its contents, carcass and liver in both sexes. The results were consistent with the passage of entecavir through the GI tract.

5. Entecavir: In vitro determination of mouse, rat, dog, monkey and human serum protein binding and/or red blood cell distribution of ^{14}C-entecavir, April 23, 1999, (Study No. 910070103)

The in vitro protein binding and red blood cell (RBC) distribution of ^{14}C-entecavir (concentrations: 50, 500 or 5000 ng/ml) were studied in fresh blood from mouse, rat, dog, monkey and human at ambient temperature. Protein binding and RBC distribution was determined by — . and liquid scintillation counting, respectively. Results: are shown in Tables 9 and 10. The extent of in vitro serum protein binding and RBC distribution of entecavir were independent of concentration and were comparable across species, except for the dog. The dog had approximately 2 to 3-fold higher protein binding compared to the other 4 species tested and negligible RBC distribution. Conclusions: the extent of in vitro serum protein binding and RBC distribution of ^{14}C-entecavir were independent of concentration and were comparable across species, except for the dog. The dog had approximately 2-3 folds higher protein binding compared to the other 4 species and negligible RBC distribution. Overall, the human data indicated that there was no potential for drug-drug interaction due to displacement of protein bound drugs when co-administered with entecavir and that entecavir was uniformly distributed in the red blood cells and plasma.

Table 9

Percent protein binding of entecavir in different species

Nominal conc. (ng/ml)	Protein binding (%)				
	mouse	rat	dog	monkey	human
50	6.2	13.8	28.1	15.2	13.8
500	8.8	11	20.3	14.5	13.9
5000	8.8	11.8	24.6	12	11.9
Mean	7.9	12.2	24.3	13.9	13.2

Table 10
Mean RBC distribution of entecavir in different species

Nominal conc. (ng/ml)	Mean RBC distribution (%)			
	Rat	dog	monkey	human
50	51.2	0	51.2	56.5
500	48.8	3.17	49.8	52.1
5000	46	1.54	48	47.5
Mean	48.7	1.57	49.7	52

2.6.4.5 Metabolism

1. One page summary report: LC/MS/MS profiling of rat bile metabolites of entecavir, 10/2/1995, (910051559)

Standard LC/MS profiling conditions and LC/MS/MS substructure analysis protocols were applied to rat bile collected from three rats at three time points (1, 2 hr and pooled). A single major metabolite corresponding to the glucuronide conjugate of entecavir was elucidated in each rat study.

2. Comparative in vitro metabolism of entecavir in liver microsomes, liver S-9, hepatocytes and liver slices from mouse, rat, dog, monkey and human, 3/17/1999, (910073839)

In vitro metabolism of entecavir was studied in liver microsomes and S-9 liver fraction from mouse, rat, dog, monkey and human; in primary hepatocytes from rat, dog and monkey; and in precision cut liver slices from rat, dog, monkey and human. Incubation was carried out with entecavir at 37 degrees C for periods ranging from 30 min up to 240 min. The initial concentration of ^{14}C-entecavir was approximately 1 μg/ml. The microsomal and S-9 fraction concentrations were 1.0 and 6.0 mg/ml in the incubation mixtures. Two liver slices, approximately 20-25 mg each were used for every 1.0 ml of incubation mixture. The metabolizing systems were determined to be active by their ability to metabolize coumarin, 7-ethoxy coumarin and/or 7-hydroxycoumarin to their respective phase 1 and/or phase 2 metabolites. The incubation mixtures were assayed for each metabolite(s) and the parent compound by HPLC radiochromatography. Results: only rat liver slices and hepatocytes metabolized entecavir to a significant extent, up to 24% in 4 hr. No metabolism of entecavir was observed in liver microsomes and S-9 fraction of any species tested (mouse, rat, dog, monkey and human) and no significant metabolism was observed in dog or monkey hepatocytes, or in dog, monkey or human liver slices. Also, in other studies not reported here, only phase 2 metabolites of entecavir were observed in rats, dogs and monkeys in vivo, and the contribution of these metabolites to the overall metabolite profile was relatively small in dogs and monkeys compared to that in rats, especially female rats. Conclusions: entecavir was not

metabolized by liver microsomal and S-9 fraction. Dog, monkey and human liver slices, and dog and monkey hepatocytes also did note metabolize entecavir to any extent. Only rat liver slices and hepatocytes metabolized entecavir to a significant extent, up to 24% in 4 hr. Entecavir glucuronide was identified as the principal metabolite. The in vitro biotransformation of entecavir is limited to only phase 2 metabolism.

3. Comparative in vivo metabolism of entecavir in rats, dogs and monkeys, 11/11/1999, (MAP007/200475)

The metabolism of ^{14}C-entecavir in male and female rats, male dogs and male monkeys was studied, and biotransformation profiles in plasma, urine and feces of the dosed animals were analyzed. The biological samples used in the current study were collected during the ADME studies. In study (MAP 178/100475/001), 4 male and 4 female rats were administered ^{14}C-entecavir intravenously or orally with a dose of 10 mg/kg. In study (MAP 178/100475/003), 3 male dogs were administered ^{14}C-entecavir intravenously at a dose of 1.0 mg/kg and orally at a dose of 2 mg/kg on two separate occasions. In study (MAP178/100475/002), 3 male monkeys were administered intravenously at a dose of 2 mg/kg and orally at a dose of 4 mg/kg. The plasma, samples were obtained and were pooled by time, gender and species. The urine and fecal samples collected at time points (ranging: 0-168 hr) from rats, dogs and monkeys were pooled separately. HPLC was utilized to study biotransformation profiling and metabolite isolation. Results: comparison of biotransformation profiles of rats, dogs and monkeys in plasma, urine and feces are shown in Tables 11, 12 and 13, respectively.

Table 11

Percent distribution of radioactive components in pooled plasma from rats, dogs and monkeys after an iv or oral dose of ^{14}C-entecavir

Sample, route	Concentration (ng-eq./ml)	Relative distribution of radioactivity in pooled samples (%)				
		M1	M2	M3	Entecavir	Others
Rat plasma, male						
1 hr-iv	3208	2	3	1	92	2
1 hr -po	3848	4	2	4	89	1
4 hr-iv	530	4	2	1	93	0
4 hr -po	985	3	1	1	81	14
6 hr-iv	163	0	5	0	83	12
6 hr-po	295	6	2	2	88	2
Rat plasma, female						
1 hr-iv	3805	4	17	3	72	4
1 hr-po	2569	4	26	4	65	1
4 hr-iv	541	0	13	3	84	0
4 hr-po	806	7	23	3	65	2
6 hr- iv	190	3	11	0	83	3
6 hr-po	270	13	17	7	59	4
Dog plasma						
1 hr-iv	129	1	0	0	98	1
1hr-po	328	1	0	0	97	2
4 hr-iv	49	1	0	0	99	0
4 hr-po	158	1	0	0	98	1
8hr-iv	20	2	0	0	95	3
8 hr-po	69	1	0	0	98	1
Monkey plasma						

1 hr-iv	189	0	4	15	81	0
1 hr-po	31	0	5	3	88	4
4 hr-iv	77	3	15	9	58	15
4 hr-po	22	2	3	5	89	1

Others= the total radioactivity in the chromatogram other than entecavir, M1, M2 and M3.

Table 12

Percent distribution of radioactive components in pooled urine from rats, dogs and monkeys after an iv or oral dose of ^{14}C-entecavir

Sample, route	Dose excreted (%)	Relative distribution of radioactivity in pooled samples (%)				
		M1	M2	M3	Entecavir	Others
Rat urine, male						
0-168 hr-iv	67.4	1	3	0	94	2
0-168 hr-po	65.7	2	3	1	89	5
Rat urine, female						
0-168 hr-iv	76.4	2	18	2	78	0
0-168 hr-po	59.2	3	29	4	63	1
Dog urine						
0-168 hr-iv	83.7	0	0	0	97	3
0-168 hr-po	67.7	1	0	0	98	1
Monkey urine						
0-168 hr-iv	76.7	1	3	2	90	4
0-168 hr-po	12.8	0	3	1	95	1

Others= the total radioactivity in the chromatogram other than entecavir, M1, M2 and M3.

Table 13

Percent distribution of radioactive components in pooled feces from rats, dogs and monkeys after an iv or oral dose of ^{14}C-entecavir

Sample, route	Dose excreted (%)	Relative distribution of radioactivity in pooled samples (%)				
		M1	M2	M3	Entecavir	Others
Rat feces, male						
0-168 hr-iv	15.3	0	1	1	96	2
0-168 hr-po	27	0	1	1	92	6
Rat feces, female						
0-168 hr-iv	20.9	0	11	4	77	8
0-168 hr-po	38.0	0	9	3	82	6
Dog feces						
0-168 hr-iv	1.4	0	0	0	94	6
0-168 hr-po	2.7	1	0	0	92	7
Monkey feces						
0-168 hr-iv	5.5	-	-	-	-	-
0-168 hr-po	77.9	1	0	3	95	1

Others= the total radioactivity in the chromatogram other than entecavir, M1, M2 and M3.

Three metabolites (M1, M2 and M3) of entecavir were found in plasma, urine and feces in male and female rats, and male monkeys, and M2 was the major metabolite. Very low

amounts of metabolites were observed in plasma, urine and feces in male dogs. No significant differences in the biotransformation profiles were observed after iv and oral administration of entecavir to the animals. The extent of metabolism of entecavir in different species was female rats > male rats> male monkeys> male dogs. The amount of metabolites as a percentage of the total radioactivity in urine or feces of the males of these animals was 10% or less and in female rats it was approximately 30%. Conclusions: only phase 2 metabolism was observed in rats, dogs and monkeys. Metabolite M1 was identified as a glucuronide conjugate and metabolite M2 and M3 were identified as sulfate conjugates of entecavir.

4. Biotransformation profiles of orally administered ^{14}C-entecavir in plasma, urine, feces, liver and lung of mice and in liver and lung of rats, 12/18/2001, (MAP023/200475)

The in vivo biotransformation of ^{14}C-entecavir was studied in mice and rats; three studies were conducted. In study #1, 12 male CD-1 mice were administered 20 mg/kg of ^{14}C-entecavir orally as a single dose. Blood, liver and lung sere collected from two mice each at 2, 4 and 8 hr and from 3 mice at 24 hr. Urine and feces (0-24) were collected from another set of 3 mice. In study #2, 8 male CD-1 mice were administered 10 mg/kg of ^{14}C-entecavir oral as a single dose and lung was collected from two mice at each 2, 4, 8 and 24 hr postdose. In study #3, nine male Sprague-Dawley rats were administered 10 mg/kg of ^{14}C-entecavir as a single dose. Blood, liver and lung were collected from two rats each at 2, 4 and 8 hr and from 3 rats at 24 hr; urine and feces (0-24 hr) were also collected from the 3 rats assigned to the 24 hr group. HPLC radiochromatographic profiles of entecavir and its metabolites were determine in plasma, urine, feces, liver and lung of mice and liver and lung of rats. Results: total radioactivity in liver and lung of rat and mouse is shown in Table 14.

Table 14
Concentration of radioactive residues in liver and lungs of mouse and rat after an iv or oral dose of ^{14}C-entecavir

Species	Radioactive residues (ng equiv/g tissue			
	2 hr	4 hr	8 hr	24 hr
Mouse				
liver	1793	749	444	713
lung	1428	524	330	304
Rat				
liver	14560	4169	3175	1101
lung	6243	1930	1060	394

In general radioactivity was high at 2 hr postdose and decreased in a time dependent manner. In mouse plasma and liver, entecavir accounted for greater than 92% of the total radioactivity. In mouse, lung, entecavir was the major radioactive component. A cluster of polar metabolites was observed in mouse lung and accounted for 5% of the radioactivity at 2 hr and 20% at 24 hr; however, these metabolites represented only

0.04% of the dose. In rat liver and lung, entecavir accounted for >90% of the total radioactivity; one liver metabolites (probably a sulfate conjugate) accounted for 6% at 2 hr. All other rat liver and lung metabolites were minor (2% or less).

Of the total excreted dose, 92% of the radioactivity was recovered in urine and 8% in feces of mice. HPLC analysis showed that entecavir was the major radioactive peak in all matrices. In mice, entecavir accounted for 92%, 95% and 71% of the radioactivity in 2 hr plasma, urine (0-24 hr) and feces (0-24 hr), respectively; most of the metabolites were very minor (<1%) and none of the metabolites was greater than 5% of the total radioactivity in that matrix. Although entecavir represented only 71% of the total radioactivity in faces, the amount of overall metabolism is relatively small considering that fecal excretion is a minor pathway.

Conclusions:

1. Nearly 95% of the radioactivity in mouse urine was parent drug. Although in 0-24 hr feces, peaks other than entecavir accounted or 29% of the radioactivity, the amount of metabolism was relatively small because fecal excretion was a minor pathway.

2. In mouse plasma (2 hr) and liver obtained 2, 4, 8 and 24 hr after dosing, entecavir accounted for greater than 92% of the radioactivity with 3-5 metabolites present; however, none of these metabolites were greater than 4%.

3. In mouse lung also, entecavir was the major radioactive component. A cluster of very early eluting, polar metabolites were present in all lung samples and its contribution increased from 5% at 2hr to 20% at 24 hr. Although the percentages of these metabolites appear to be large, the absolute amount is very small and at 24 hr is less than 0.04% of the initial dose.

4. In rat lung and liver, entecavir accounted for greater than 90% of the radioactivity at all time points. Polar metabolites seen in mouse lung were not observed in rat lung, but a small amount of polar metabolites (0.2 – 1.6% of the total radioactivity in liver) was observed in rat liver.

5. Following oral administration to mice and rats, entecavir was excreted largely unchanged and no major metabolites were observed.

2.6.4.6 Excretion

1. Mass balance of total radioactivity and pharmacokinetic of entecavir in rats following intravenous and oral administration of ^{14}C-entecavir, 8/11/1999, (MAP003/200475)

The mass balance of total radioactivity and pharmacokinetics of entecavir in rats were determined in a parallel design study in which one group of 8 rats (4/gender) received a single iv (10 mg/kg) dose of ^{14}C-entecavir as a 5-minute zero order iv infusion and

another group of 8 rats (4/gender) received oral (10 mg/kg) doses of ^{14}C-entecavir. Blood, urine and feces were obtained over 7 days and were analyzed by a validated LC/MS/MS method. Results: mean pharmacokinetic parameters are shown in Table 15.

Table 15
Single dose pharmacokinetics of ^{14}C-entecavir in rats after iv (10 mg/kg) or po (10 mg/kg) administration.

Route	Cl (ml/min/kg)	Cmax (μg/ml)	AUCinf (μg*hr/ml)	T1/2 (hr)	Vdss (l/kg)	Fecal Rec (%)	Urinary Rec (%)
IV	22.8	3.63	3.76	2.06	4.63	-	50.5
PO	25.1	1.12	3.11	2.2	-	-	44
Total radioactivity							
IV	-	5.15	6.77	2.03	-	18.1	71.9
PO	-	1.72	6.50	2.32	-	32.5	62.5

Overall recovery of radioactivity within 7 days following the iv and po doses was 92.5% and 96.9%, respectively. Approximately 71.9% and 18.1% of the administered radioactivity after the iv dose, and 62.5% and 32.5% of the administered radioactivity after the po dose were recovered in the urine and feces, respectively. Following administration by either route, most of the radioactivity was recovered within 24 hr in the urine and feces; unchanged entecavir accounted for majority of the radioactivity in the urine. The extent of absorption, first pass metabolism and oral bioavailability of entecavir were 86.9%, 8.4% and 79.6% in the rat, respectively.

Conclusions:

1. Following po administration of a single 10 mg/kg dose of ^{14}C-entecavir, the extent of absorption of the oral dose was essential complete (86.9%) and the absolute bioavailability was 79.6%

2. Following iv administration of a single 10 mg/kg dose of ^{14}C-entecavir, the Vdss of entecavir was 4.63 L/kg which suggested extensive extravascular distribution of drug.

3. Following iv and po doses of ^{14}C-entecavir, plasma levels of total radioactivity were slightly higher than those of entecavir, suggesting the presence of some metabolite(s) of entecavir in systemic circulation; first pass metabolism of entecavir was minimal (8.4%).

4. Following iv or po doses of ^{14}C-entecavir, the plasma concentrations of entecavir declined rapidly with half-life of 2.1 to 2.2 hr after both iv and po dosing.

5. Approximately 71.9% and 18.1% of the administered radioactivity following the

iv dose and 62.5% and 32.5% of the administered radioactivity after the po dose were recovered within 7 days in the urine and feces, respectively. Unchanged entecavir accounted for majority of the radioactivity in the urine, indicating that renal elimination played a predominant role in the disposition of entecavir.

2. Summary report: Limited evaluation-biliary and urinary excretion of entecavir after iv administration to the rat, 11/09/1995, (Report No. 910051407)

Entecavir was administered iv to 3 male rats at single dose levels of 2550, 2640 or 2570 µg/rat to determine if biliary excretion of parent drug is a major route of elimination in the rat. Bile and urine samples were collected at hourly intervals for 10 hr and were analyzed by a validated LC/MS method to determine the concentration of unchanged entecavir. Results: are summarized in Table 16.

Table 16
Bile profile of entecavir in male rats after iv administration.

Treatment		Bile		Urine		Total
Rat #	Dose (µg)	Amount (µg)	% of dose	Amount (µg)	% of dose	% of dose
1						
2						
3						

The mean percent of the administered dose excreted in the bile was <0.5%; whereas, the mean percent of the dose excreted in the urine as unchanged drug was approximately 68%. Conclusions: these results demonstrated that the rat biliary excretion of entecavir was not a major route of elimination and that urinary excretion of unchanged drug appeared to be the primary route of elimination.

3. Mass balance of total radioactivity and pharmacokinetics of entecavir in dogs following iv and oral administration of ^{14}C-entecavir, 11/8/1999, (Study # 920001977)

Three male beagle dogs received single iv (1 mg/kg) and oral (2 mg/kg) doses of ^{14}C-entecavir in a crossover design study to determine the mass balance and pharmacokinetics. There was a 2 weeks washout period between the two treatments. Blood, urine and feces were obtained and were analyzed by a validated LC/MS/MS method. Results: mean pharmacokinetic parameters are shown in Table 17.

Table 17

Single dose pharmacokinetics of ^{14}C-entecavir in male dogs after iv (1 mg/kg) or po (2 mg/kg) administration.

Route	Cl (ml/min/kg	Cmax (µg/ml)	AUC (µg*hr/ml)	T1/2 (hr)	Vdss (l/kg)	Fecal Rec (%)	Urinary Rec (%)
IV	8.6	3.0	1.89	8.95	1.6	-	-
PO	-	1.57	4.3	22.7	-	-	-
Total radioactivity							
IV	-	3.3	2	1.65	-	1.4	83.7
PO	-	1.7	4.3	1.97	-	2.69	67.7

Cmax and AUC values indicated that most of the circulating plasma radioactivity was accounted for by unchanged drug. A trace amount of a single metabolite was found in pooled urine. This implied that most of the radioactivity recovered in urine was presumably unchanged drug. Overall, recovery of radioactivity (urine+feces+case rinse, wash, wipes and debris) within 7 days following the iv and po doses was 92.4% and 88.3%, respectively. The extent of absorption, first pass metabolism and oral bioavailability of entecavir were 109%, 0.47% and 118%, respectively. Conclusions: entecavir was completely absorbed after oral administration to dogs, underwent minimal first pass metabolism, distributed extravascularly and was eliminated predominantly by the renal route, mainly as unchanged drug, following either iv or po administrations. Overall, recovery of radioactivity within 7 days following the iv and po doses of ^{14}C-entecavir was 92.4% and 88.3%, respectively. Approximately 83.7% and 1.4% of the administered radioactivity after the iv dose and 67.7% and 2.69% of the administered radioactivity after the po dose were recovered in the urine and feces, respectively. Unchanged entecavir accounted for an appreciable amount of radioactivity in the urine, indicating that renal elimination plays a predominant role in the disposition of entecavir.

4. Mass balance of total radioactivity and pharmacokinetics of entecavir in monkeys following iv and oral administration of ^{14}C-entecavir, 11/15/1999, (Study # 920001978)

Three male monkeys received a single iv (2 mg/kg) and oral (4 mg/kg) doses of ^{14}C-entecavir in a crossover design study to determine the mass balance and pharmacokinetics in monkeys. There was a 2 week washout period between the treatments. Blood, urine and feces were obtained and were analyzed by a validated LC/MS/MS method. Results: mean pharmacokinetic parameters are shown in Table 18.

Table 18

Single dose pharmacokinetics of ^{14}C-entecavir in male monkeys after iv (2 mg/kg) or po (4 mg/kg) administration.

Route	Cl (ml/min/kg	Cmax (µg/ml)	AUC (µg*hr/ml)	T1/2 (hr)	Vdss (l/kg)	Fecal Rec (%)	Urinary Rec (%)
IV	11.2	12.0	3.3	5.98	1.02	-	59.8
PO	-	0.12	0.1	27.4	-	-	11.8
Total radioactivity							
IV	-	15.7	3.6	0.98	-	5.5	76.7
PO	-	0.15	0.49	-	-	77.9	12.8

Most of the urinary and fecal radioactivity was recovered within 48 hr after dosing; unchanged entecavir accounted for the majority of the radioactivity in the urine. Overall recovery of radioactivity (urine+feces+case rinse, wash, wipes and debris) within 7 days following the iv and po doses was 89.5% and 94.6%, respectively. The extent of absorption, first pass metabolism and oral bioavailability of entecavir were 16.6%, 11.5% and 16.1%, respectively. Overall, the results indicated that entecavir was incompletely absorbed, with a bioavailability of 16.1% after po administration, underwent minimal first pass metabolism, was distributed extravascularly, and was eliminated predominantly by the renal route, mainly as unchanged drug, following either iv or po administration to monkeys.

Conclusions: entecavir was incompletely absorbed (bioavailability = 16.1%) after oral administration to monkeys, underwent minimal first pass metabolism, distributed extravascularly and was eliminated predominantly by the renal route, mainly as unchanged drug, following either iv or po administration.

Overall, recovery of radioactivity within 7 days following the iv and po doses of ^{14}C-entecavir was 89.5% and 94.6%, respectively. Approximately 76.7% and 5.48% of the administered radioactivity following the iv dose and 12.8% and 77.9% of the administered radioactivity after the po dose were recovered within 7 days in the urine and feces, respectively. Unchanged entecavir accounted for majority of the radioactivity in the urine, indicating that renal elimination played a predominant role in the disposition of entecavir in monkeys.

2.6.4.9 Discussion and Conclusions

The in vitro and in vivo studies were conducted to determine the systemic exposure and to investigate the ADME of entecavir in the species routinely used for toxicological assessment (mice, rats, rabbits, dogs, and monkeys). Additional studies were conducted in the woodchuck and the duck, which were used as animal models of anti-HBV activity. TK evaluations of entecavir, in support of pivotal toxicology studies, were conducted in compliance with GLP regulations.

The monohydrate of entecavir, also known as BMS-200475, was used in all studies unless otherwise specified. All doses used in the nonclinical in vivo studies are presented in terms of the free base. If radiolabeled compound was used, the radiochemical purity of the materials used in the various nonclinical studies was > 97%. Oral doses of entecavir were administered as an aqueous solution or suspension (water or saline), a suspension in 1% Avicel/0.25% Methocel or in 1% sodium carboxymethylcellulose (CMC), as bulk drug in gelatin capsules, or in ground rodent diet. Intravenous doses of entecavir were administered in sterile water or saline as a solution.

PK studies demonstrated that entecavir was rapidly absorbed following oral administration to rats and dogs. The absorption was slower in monkeys relative to rats and dogs. Monkey also had lower bioavailability compared to rats and dogs. The rate of absorption in mice, woodchucks, rabbits, and ducks was also rapid. The absolute oral bioavailability of entecavir was high (>80%) in rats and dogs, and lower (~16%) in monkeys. The low bioavailability in monkeys was due to poor absorption and was not related to first-pass metabolism.

Dose-related systemic exposure to entecavir was established after multiple doses in species used for nonclinical toxicology testing. In some studies, the exposure increased in proportions similar to the dose increment. In other studies the exposure was dose-related but not in proportion to the dose increment. In other studies the exposure was dose-related but not in proportion to the dose increment. Mean steady-state exposures (combined sex except for rats) in toxicology studies ranged up to approximately 4550 and 2560 times the exposures in humans at 0.5 (~15 ng*h/mL) and 1.0 mg (~30 ng*h/mL), respectively. There were no sex-related differences in mice, dogs, monkeys, or humans. Exposure to entecavir was higher in male rats than in female rats. The biotransformation results indicated that the difference in exposure to entecavir in male and female rats was attributable to the greater production of the major metabolite, M2, in female rats. Entecavir was also found in the CSF in mice, rats, dogs, and monkeys, indicating that entecavir can cross the blood/brain barrier. The systemic exposure to entecavir, at doses producing good antiviral activity in woodchucks and ducks, was lower than the exposures observed in the toxicological assessment of entecavir in mice, rats, rabbits, dogs, and monkeys.

The terminal plasma elimination T-half of entecavir, following IV administration, ranged from approximately 2 to 9 h in selected species. In comparison, the mean terminal phase plasma T-half of entecavir was approximately 130 h in humans. The marked difference in the T-half values between animals and humans is probably related to the longer sampling scheme and more sensitive assays used in human studies. Values for the systemic clearance (CLT) of entecavir in rats, dogs, and monkeys ranged from 8.6 to 46.7 mL/min/kg. In humans, the estimated value for entecavir CLT was somewhat lower than in the animal species (approximately 6-7 mL/min/kg based on an estimated bioavailability of 70 to 80%). There was no indication of systemic accumulation of entecavir after repeated administration in rats and monkeys, although there was low to moderate accumulation in dogs (1.1- to 2.5-fold increase). In humans the systemic accumulation ratio of entecavir ranged from 1.6 to 2.7 in the dose range of 0.1 to 1.0

mg/day, which was similar to the accumulation observed in dogs. The human accumulation ratios are indicative of an effective T-half of ~ 24 h, although the terminal phase plasma T-half in humans was approximately 130 h. Additionally, steady state entecavir PK in humans are dose-linear over the dose range of 0.1 to 1.0 mg.

Values for the VSS in rats (4.6 L/kg), dogs (1.6 L/kg), and monkeys (1.0 L/kg) were greater than the volume of total body water in these species (~0.7 L/kg), suggesting extensive extravascular distribution of the drug and/or preferential binding in tissues. Similarly, the apparent volume of distribution in humans was also large. Tissue distribution studies in mice and rats indicated that entecavir was rapidly and extensively distributed throughout the body with no sex differences in tissue distribution. The highest percentages of dosed radioactivity were associated with the gastrointestinal (GI) tract tissues, liver, and kidney. This is not surprising since entecavir was dosed orally and was mainly eliminated in the urine. The distribution of radioactivity to the liver indicates that entecavir is able to reach its intended site of action. The elimination of radioactivity occurred rapidly, with most of the administered radioactivity recovered in 24 hours. Drug-related radioactivity was widely distributed in maternal and fetal tissues of pregnant rats, and was secreted in the milk of lactating rats within 1.0 h after oral administration of [14C]-entecavir. These findings indicate the potential for fetal and neonatal exposure to entecavir and/or its metabolites if the drug is administered to pregnant or lactating women. In addition, [14C]-entecavir derived radioactivity was detected in maternal cerebrum in pregnant rats, and lower (compared to concentrations in plasma) but dose-related increases in exposure to entecavir were found in the CSF of mice, rats, dogs, and monkeys in other TK studies, findings further support that entecavir can cross the blood/brain barrier.

In vitro, the serum protein binding of entecavir was low in mouse, rat, dog, monkey, and human (ranging from 7.9 to 24%), and entecavir was uniformly distributed between plasma and red blood cells (RBC) in human blood. The low protein binding in humans (13%) suggests that there is little potential for drug interactions due to displacement of highly protein bound drugs when co-administered with entecavir. Protein binding was independent of serum drug concentrations within the entecavir concentration range of 50 to 5000 ng/mL.

Entecavir is eliminated mainly as unchanged drug in the urine. Biliary clearance plays a very minor role. The majority of the drug related components in feces was unchanged entecavir. Only Phase II metabolites of entecavir were found in rats, dogs, monkeys, and humans. Two sulfate conjugates (M2 and M3) and 3 glucuronide conjugates (M1, M4, and M5) of entecavir were detected in male and female rats and male monkeys. In dogs, only the glucuronides M1, M4, and M5 of entecavir were detected. In humans, four Phase II metabolites of entecavir were detected, namely, M1, M2, M4, and M5. Thus, all of the metabolites identified in humans were present in the species used in the toxicological evaluation of entecavir. The total amount of metabolites, as a percentage of the total radioactivity in urine or feces, was approximately 10% or less in male rats and approximately 30% in female rats. The metabolite M2 was the major metabolite in female rats, accounting for the main difference in the extent of metabolism in male and

female rats (ie, metabolites constituted a higher percentage of the total radioactivity in plasma, urine, and feces in female rats). Consistent with these in vivo findings, in vitro biotransformation studies indicated that there was no significant metabolism of entecavir in mouse, rat, dog, monkey, and human liver microsomes or S9 fraction; dog and monkey hepatocytes; and dog, monkey, and human liver slices. Only rat hepatocytes and rat liver slices metabolized entecavir to an appreciable extent. Metabolite M1 was the only metabolite identified in rat hepatocytes and liver slices. The biotransformation of [14C]-entecavir was also investigated in mouse lung microsomes, mouse and rat lung homogenates, and mouse and rat lung S9 fraction. Entecavir was not metabolized by mouse lung microsomes, or by mouse and rat lung homogenates and lung S9 fraction. No oxidative metabolism was detected in any of these in vitro studies.

Entecavir, at concentrations up to 300 μM, was not an inhibitor of the major human cytochrome P450 (CYP) isoforms (1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4) involved in drug metabolism. This finding indicates that entecavir has little potential to inhibit the metabolic clearance of drugs that are highly metabolized by the major human CYP isozymes. In addition, entecavir, at concentrations up to 10μM, did not seem to induce the major human CYP isoforms (1A2, 2B6, 2C9, 2C19, and 3A4/5). Overall, the in vitro findings indicate that entecavir is not a substrate, inhibitor or inducer of the CYP isozymes, and that the PK of entecavir in humans is unlikely to be affected by the co-administration of agents that are either metabolized by, inhibit, or induce the CYP system. Likewise, the PK of known CYP substrates are unlikely to be affected by the co-administration of entecavir.

Mass balance studies in rats, dogs, and monkeys indicated that [14C]-entecavir was eliminated in the urine and feces. In rats and dogs, after either IV or oral administration, the excretion of radioactivity (as a % of dose) ranged from approximately 63% to 84% and 1.4% to 33% in the urine and feces, respectively; the overall recovery ranged from 88% to 97%. In monkeys, the excretion of radioactivity was 77% and 5.5% in the urine and feces, respectively, after an IV dose, while it was 13% and 78% in the urine and feces, respectively, after an oral dose. Overall recovery of radioactivity following IV and oral doses was 90% and 95%, respectively. In these animal studies, unchanged entecavir accounted for the majority of radioactivity recovered in the urine. In a single PO dose (1.0 mg) study of [14C]-entecavir in humans, the mean urinary recovery of entecavir and radioactivity over 336 h accounted for approximately 70% and 76%, respectively, and fecal recovery of radioactivity over 336 h accounted for approximately 6% of the PO dose. Thus, of the total radioactivity excreted in urine, approximately 90% was entecavir. In rats, monkeys, and humans, the values of renal clearance are greater than the values of glomerular filtration rate (GFR), suggesting that entecavir is eliminated through a combination of passive (glomerular filtration) and active (net tubular secretion) processes by the kidney.

In conclusion, entecavir is highly bioavailable in various animal species and humans although it has lower bioavailability in monkeys. High multiples of human exposures were achieved in animal species utilized in toxicology studies. The steady state volume of distribution of entecavir in animals was greater than the volume of total body water, suggesting extravascular distribution of the drug and/or preferential binding in tissues.

Low serum protein binding was demonstrated in all species. In rats, dogs, monkeys, and humans, only Phase II conjugation of the parent drug was observed, resulting in the formation of glucuronide (M1, M4, and M5) and sulfate (M2 and M3) metabolites. Entecavir is not a substrate, inhibitor, or inducer of the CYP isozymes. Renal clearance of the drug plays a predominant role in the elimination of entecavir with parent drug as the major form present in urine. In rats, monkeys, and humans, the rate of renal clearance is greater than GFR indicating that renal elimination occurs via a combination of glomerular filtration and net tubular secretion. Overall, the absorption, distribution, metabolism, and excretion profiles of entecavir in mice, rats, dogs, and monkeys, compared to humans, suggest that these species were appropriate for the safety assessment of entecavir and its metabolites.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The following tables and figures were constructed by the sponsor:

Single dose pharmacokinetics of entecavir

Species		**Mouse / CD-1, and C57BL/6**					
Sex (M/F) / Number of Animals		M15 / dose / strain					
Feeding condition		Non-fasted					
Vehicle/Formulation		Water, solution					
Method of Administration		Single (PO)					
Dose (mg/kg)		4 and 10					
Sample(s)		Plasma					
Analyte(s)		ETV					
Assay(s)		HPLC/MS/MS					
Strain	**Dose (mg/kg)**	**Cmax (ng/mL)**	**Tmax (h)**	**AUC(0-24) (ng•h/mL)**	**Dose Ratio**	**Cmax Ratio**	**AUC(0-24) Ratio**
CD-1	4	462	0 50	840	1.0	1.0	1.0
	10	1507	0 50	2877	2 5	3 3	3.4
C57BL/6	4	1100	0 50	1389	1 0	1 0	1.0
	10	3414	0.50	3480	2 5	3 1	2.5

	Test Article: ETV
	Document Control No. 910050874
Species	**Rat**
Sex (M/F) / Number of Animals	M3 / dose
Feeding condition	--[a]
Vehicle/Formulation	Sterile water, solution
Method of Administration	Single (PO), single (IV)
Dose (mg/kg)	25 (PO), 10 (IV)
Sample(s)	Plasma
Analyte(s)	ETV
Assay(s)	LC/MS

Route	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-24) (ng•h/mL)	T-half (h)	CLT (mL/min/kg)	VSS (L/kg)	F %
PO (N=3)	25	1220	1.5	5470	5.2	--	--	37
IV (N=3)	10	7810	0.16	5920	4.0	28.1	3.8	--

	Test Article: ETV
	Document Control No. 910064656
Species	**Woodchuck**
Sex (M/F) / Number of Animals	F2
Feeding condition	Non-fasted
Vehicle/Formulation	Liquid diet, mixture
Method of Administration	Single PO
Dose (mg/kg)	0.5
Sample(s)	Plasma
Analyte(s)	ETV
Assay(s)	LC/MS/MS

Mean Pharmacokinetic Parameter Values (N=2)

Dose (mg/kg)	Cmax (ng/mL)	Tmax[a] (h)	T-half (h)	AUC(INF) (ng•h/mL)
0.5	17.09	1.50 (1.00, 2.00)	4.27	73.97

Species	Dog/Beagle										
Mean (SD) Pharmacokinetic Parameter Values for Total Radioactivity											
Route	**Cmax (ng/mL)**	**Tmax (h)***	**AUC(INF) (ng•h/mL)[a]**	**T-half (h)**	**%F**	**CLT**	**CLR**	**%UR**	**%FR**	**VSS (L/kg)**	**Total Recovery**
						(mL/min/kg)					
PO (N=3)	1704 (442)	0.50	4313 (571)	1.97 (0.28)	NA	NA	NA	67.7 (7.49)	2.69 (1.02)	NA	88.3
IV (N=83	3358 (167)	0.16	2012 (199)	1.65 (0.12)	NA	NA	NA	83.7 (17.6)	1.40 (0.80)	NA	92.4
Mean (SD) Pharmacokinetic Parameter Values for ETV											
PO (N=3)	1575 (403)	0.25	4365 (739)	22.7 (8.35)	118 (8.60)	NA	3.09 (0.78)	ND	ND	NA	NA
IV (N=3)	3065 (43.3)	0.10	1890 (310)	8.95 (7.17)	NA	8.60 (1.48)	3.82 (1.22)	ND	ND	1.60 (0.51)	NA

* Median (minimum, maximum) reported, NA: Not applicable, ND: Not determined, FR: Fecal recovery

Species	Monkey/Cynomolgus										
Mean (SD) Pharmacokinetic Parameter Values for ETV											
Route	**Cmax (ng/mL)**	**Tmax (h)[a]**	**AUC(INF) (ng•h/mL)**	**T-half (h)**	**%F**	**CLT**	**CLR**	**%UR**	**%FR**	**VSS (L/kg)**	**Total Recovery**
						(mL/min/kg)					
PO (N=3)	124 (78)	2.00	1013 (84.7)	27.4 (21.3)	16.1	NA	10.5 (8.29)	11.8 (6.85)	ND	--	NA
IV (N=3)	12078 (5790)	0.10	3645 (1935)	5.98 (7.51)	NA	11.2 (4.85)	6.63 (3.09)	59.8 (6.88)	ND	1.02 (0.73)	NA
Mean (SD) Pharmacokinetic Parameter Values for Total Radioactivity											
PO (N=3)	150 (100)	2.00	490[b] (320)	--[c]	NA	NA	NA	12.8 (3.97)	77.9 (1.79)	NA	94.6
IV (N=3)	15740 (8350)	0.10	3630 (1780)	0.98 (0.29)	NA	NA	NA	76.7 (7.03)	5.48 (1.01)	NA	89.5

[a] Median (minimum, maximum) reported, [b] AUC(0-T) reported, [c] Not reported due to the lack of a log-linear terminal phase in the plasma profiles
NA: Not applicable, ND: Not determined

Multiple dose pharmacokinetics of entecavir

				Test Article:	ETV
				Document Control No.	910070306
Species		**Mouse/ CD-1**			
Sex (M/F) / Number of Animals		M10 / dose, F10 / dose			
Feeding condition		Non-fasted			
Vehicle/Formulation		Water, solution			
Method of Administration		Multiple PO			
Dose (mg/kg/day)		0.2, 1, 5			
Sample(s)		Plasma, CSF			
Analyte(s)		ETV			
Assay(s)		LC/MS/MS			
Dose (mg/kg/day)	**Study Day**	**Cmax (ng/mL)**		**AUC(0-24) (ng•h/mL)**	
		Male	**Female**	**Male**	**Female**
0.2	58	69.4	56.7	321	336
1	58	207	146	313	1207
5	58	764	1091	1187	1271

				Test Article:	ETV
				Document Control No.	910070460
Species		**Rat/Sprague-Dawley**			
Sex (M/F) / Number of Animals		M10 / dose, F10 / dose			
Feeding condition		Non-fasting			
Vehicle/Formulation		water, solution			
Method of Administration		Multiple PO			
Dose (mg/kg/day)		0.6, 3, 15			
Sample(s)		Plasma, CSF			
Analyte(s)		ETV			
Assay(s)		LC/MS/MS			
Dose (mg/kg/day)	**Study Day**	**Cmax (ng/mL)**		**AUC(0-24) (ng•h/mL)**	
		Male	**Female**	**Male**	**Female**
0.6	1	61.4	61.5	257	170
	85	72.9	40.1	477	420
	176	90.2	46.0	408	257
3	1	428	364	1413	1146
	85	410	255	2396	1217
	176	399	211	2151	998
15	1	2144	1620	8977	5668
	85	1262	577	7941	2483
	176	1141	984	6318	4504

Test Article:	ETV
Document Control No.	910062409
Species	**Dog/Beagle**
Sex (M/F) / Number of Animals	M3 / dose, F3 / dose
Feeding condition	fasted
Vehicle/Formulation	Dry-filled gelatin capsule
Method of Administration	Multiple PO
Dose (mg/kg/day)	1, 10, 100 (lowered to 50 on day 6)
Sample(s)	Plasma
Analyte(s)	ETV
Assay(s)	HPLC/fluorescence

Mean (SD) Pharmacokinetic Parameter Values for ETV

Dose (mg/kg/day)	Study Day	Cmax (ng/mL)		AUC(0-24) (ng•h/mL)	
		Male	Female	Male	Female
1	1	805 (38)	939 (125)	2791 (354)	3259 (604)
	9	913 (386)	1051 (225)	3924 (1615)	5547 (1011)
10	1	7988 (440)	9104 (841)	50759 (4737)	52102 (6719)
	9	10760 (305)	12135 (190)	105894 (36103)	54679 (2772)
100/50[a]	1	72698 (24189)	46790 (7036)	864207 (350490)	562293 (18960)
	9	[b]	[b]	[b]	[b]

[a] 100 mg/kg/day lowered to 50 mg/kg/day on Day 6, [b] N=1

Test Article:	ETV
Document Control No.	910058834
Species	**Monkey/Cynomolgus**
Number of Animals	3 / dose
Feeding condition	--[a]
Vehicle/Formulation	1% sodium carboxymethylcellulose (CMC), suspension
Method of Administration	Multiple PO
Dose (mg/kg/day)	5, 25
Sample(s)	Plasma
Analyte(s)	ETV

Mean (SD) Pharmacokinetic Parameter Values for ETV N=3

Dose (mg/kg/day)	Study Day	Cmax (ng/mL)	AUC(0-8) (ng•h/mL)
5	1	149	416
	29	186	655
25	1	585	2226
	29	578	2246

Tissue Distribution of entecavir

Test Article:	[^{14}C]-ETV
Document Control No.:	920001980
Species:	Mouse/ CD-1 outbred albino

Mean Concentration(ng equiv/g) of [^{14}C]-ETV, N=3

Tissue/Organ	0.5 h		4 h		24 h		48 h		96 h		168 h	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Bladder	127966	5848	812	396	193	159	168	158	127	162	67.5	93.2
Blood	2586	3170	96.3	97.9	13.7	8.65	5.74	4.79	2.30	2.98	2.76	2.47
Bone	3143	2550	352	188	72.7	5.15	39.2	9.44	58.7	6.45	41.8	2.12
Brain	180	214	119	106	66.9	61.3	51.7	57.8	36.8	35.5	19.6	18.9
Carcass	4574	2591	342	234	164	83.6	136	74.7	115	59.0	224	62.4
Eyes (both)	810	725	133	171	53.7	63.9	27.9	36.9	21.6	14.2	25.4	21.4
Heart	3098	3915	432	489	331	366	228	301	123	197	67.9	113
Kidneys	24471	39003	1313	1495	704	483	515	390	374	352	334	262
Large intestine	2407	2035	11993	14939	116	90.5	52.8	18.5	33.1	1.52	21.6	0
Liver	3787	2920	733	538	255	196	268	181	240	160	165	107
Lungs	3024	3456	390	390	152	162	106	107	75.0	84.6	52.1	62.4
Muscle	3385	3624	239	238	163	153	149	149	127	142	122	132
Ovaries	NA	2229	NA	323	NA	240	NA	119	NA	77.2	NA	69.5
Skin	3688	3721	241	216	75.3	73.2	56.3	39.7	46.5	41.5	42.4	44.3
Small intestine	21638	7881	940	734	121	89.4	80.5	41.5	40.1	17.8	36.1	9.72
Spleen	16947	23456	2218	2240	316	376	202	239	128	150	89.6	90.0
Stomach	57671	20017	681	738	56.7	24.2	56.5	11.5	24.5	0.1	26.8	0
Testes	2216	NA	395	NA	167	NA	117	NA	74.1	NA	40.1	NA

NA = not applicable

APPEARS THIS WAY
ON ORIGINAL

Protein binding of entecavir

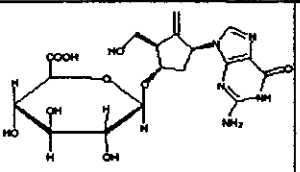
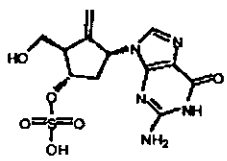
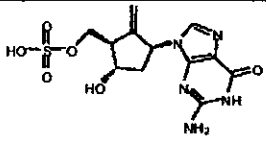
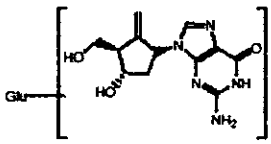
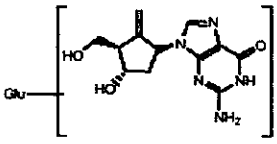
			Test Article:	[^{14}C]-ETV	
			Document Control No.	910070103	
Study system:	*In vitro* serum				
Analyte, Test system and Analytic method:	ETV, [redacted], Liquid Scintillation Spectrometry				
Species	**ETV Concentration ng/mL**	**% Bound (N=3)***	**Overall Mean**	**Document Control No.**	**Location in Dossier**
Mouse	50	6.2	7.9	910070103	
	500	8.8			
	5000	8.8			
Rat	50	13.8	12.2		
	500	11.0			
	5000	11.8			
Dog	50	28.1	24.3		
	500	20.3			
	5000	24.6			
Monkey	50	15.2	13.9		
	500	14.5			
	5000	12.0			
Human	50	13.8	13.2		
	500	13.9			
	5000	11.9			

* conducted in triplicate/concentration; protein binding determined from a mean serum value and a pooled [redacted] value; protein binding value not corrected for non-specific binding (≤1.3%)

APPEARS THIS WAY
ON ORIGINAL

Metabolism of entecavir in vivo:

Structures of entecavir metabolites

Test Article: ETV					
Metabolite	**Structure[a]**	**CTD Table**	**Metabolite**	**Structure[a]**	**CTD Table**
M1		2.6.5.8B 2.6.5.8C 2.6.5.9B	M3		2.6.5.8B 2.6.5.8C 2.6.5.9B
M2		2.6.5.8B 2.6.5.8C 2.6.5.9B	M4		2.6.5.8B 2.6.5.8C 2.6.5.9B
M5		2.6.5.8B 2.6.5.8C 2.6.5.9B			

a) The structures of the metabolites M1 and M2 were based on MS fragmentation pattern and NMR. The structure of M3 was based on MS fragmentation pattern only. The structures of metabolites M4 and M5 were based on MS - SRM data only. Glu indicates glucuronide conjugation in metabolites M4 and M5

Comparative biotransformation of [14C]-entecavir in mice and rats

Test Article: [^{14}C]-ETV								
Mice								
Route	**Sample**	**Sampling Time**	**Percentage of Cumulative Dose in Sample**	**Relative Distribution of ETV and Its Metabolites[a] (%)**				
				Parent	**M1**	**M2**	**M3**	**Others[c]**
PO	Plasma[b]	2 h	NA	91.7	ND	ND	ND	8.3
	Urine[b]	0-24 h	NR	94.6	ND	ND	ND	5.4
	Feces[b]	0-24 h	NR	70.9	ND	ND	ND	29.8
Rats - Male								
Route	**Sample**	**Sampling Time**	**Percentage of Cumulative Dose in Sample**	**Relative Distribution of ETV and Its Metabolites[a] (%)**				
				Parent	**M1**	**M2**	**M3**	**Others[c]**
IV	Plasma[b]	1 h	NA	92	2	3	1	2
		4 h	NA	93	4	2	1	ND
		6 h	NA	83	ND	5	ND	12
	Urine[b]	0-168 h	67	94	1	3	ND	2
	Feces[b]	0-168 h	15	96	ND	1	1	2
PO	Plasma[b]	1 h	NA	89	4	2	4	1
		4 h	NA	81	3	1	1	14
		6 h	NA	88	6	2	2	2
	Urine[b]	0-168 h	66	89	2	3	1	5
	Feces[b]	0-168 h	27	92	ND	1	1	6

Comparative biotransformation of [14C]-entecavir in humans

Human[d]										
Route	Sample	Sampling Time	Percentage of Cumulative Dose in Sample	Relative Distribution of ETV and Its Metabolites[a] (%)						
				Parent	M1	M2	M3	M4	M5	Others[c]
PO	Plasma[b]	1 h	NA	79	9	ND	ND	6	6	1
		2 h	NA	70	20	ND	ND	4	5	1
	Urine[b]	0-336 h	76	87	6	ND	ND	ND	4	3
	Feces[b]	0-336 h	6	66	N/D	15	ND	ND	ND	19

[a] The structure of the metabolites were determined based on LC/MS analysis of the fragmentation pattern and in some cases NMR analysis. In some matrices, the metabolite identification was solely based on comparison of HPLC retention time with previously characterized metabolites and co-elution with spiked metabolite standards. The structure of the human metabolites M4 and M5 were assigned based on LC/MS data and data from hydrolysis of M5 with β-glucuronidase.

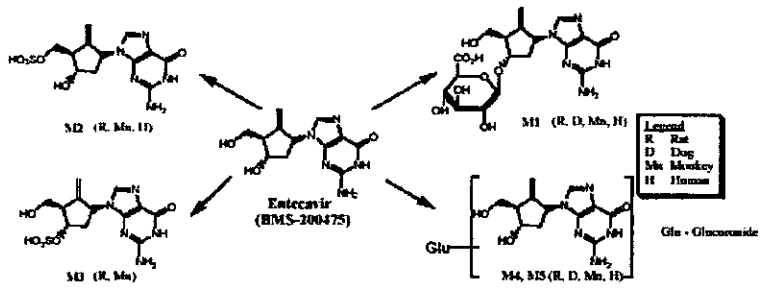
[b] Relative distribution of metabolites in these plasma, urine, and fecal samples are the percent distribution of radioactive metabolites in the extracts of these samples.

[c] Others: radioactive components other than ETV, M1, M2, M3, M4, and M5

[d] In the LC/MS/MS conditions used for the analysis of rat, dog, and monkey samples only M1, M2, M3, and ETV were detected. The LC/MS/MS conditions employed for the analysis of human samples were different from the one employed for the analysis of rat, dog, and monkey samples and under these conditions metabolites M1, M4, M5, and ETV were detected in human plasma, while only metabolites M1, M5, and ETV were detected in human urine. When the same LC/MS/MS condition was used for analysis of rat, dog, and monkey plasma samples from a different study (Table 2 6.5 8C) metabolites M1, M4, and M5 were also detected.

ND: Not detected
NA: Not applicable
NP: Not processed due to low amounts of radioactivity
NR: Not reported

Major metabolic pathways of entecavir



Note: The metabolism of ETV to M1, M4 and M5 is probably mediated by UDP-glucuronyl transferases, and metabolism to M2 and M3 is probably mediated by sulfotransferases.

Excretion: urinary and fecal excretion after a single po and single iv dose

		Test Article: ETV
		Document Control Number: 910059456
Species/strain		**Dog/Beagle**
Sex (M/F)/Number of animals		M/3
Feeding condition		fasted
Vehicle/Formulation		Sterile water, pH 9 (PO), sterile saline (IV)
Method of Administration		Single PO, IV
Dose (mg/kg)		10, 25 (PO), 5, 10 (IV)
Analyte(s)		ETV
Assay(s)		HPLC, LC/MS/MS
		Mean % Recovery of Urine 0-24 h N=3
Route	**Dose mg/kg**	**% Urine**
PO	10	63.2
	25	82.9
IV	5	72.3
	10	76.0

Biliary and urinary excretion after a single iv dose

				Test Article:	ETV	
				Document Control Number:	910051407	
Species/strain		**Rat**				
Sex (M/F)/Number of animals		M/3				
Feeding condition		[a]				
Vehicle/Formulation		Sterile water, pH 9				
Method of Administration		Single IV				
Dose (mg/kg)		10				
Analyte(s)		ETV				
Assay(s)		LC/MS/MS				
		Bile		**Urine**		**Total**
Rat #	**Dose (mg)**	**Amount (mg)**	**% of Dose**	**Amount (mg)**	**% of Dose**	**% of Dose**
425	2.55					
426	2.64					
427	2.57					

[a] Not available

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Single-Dose Toxicity

1. Mice: Groups of male and female mice (5 animals/sex/group) received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high). Key findings: beginning day 3, dose-related, transient, minimal to marked body weight losses were noted (mid, high and very high). Dyspnea, collapse and death were noted in one male mouse (high). Overt signs of toxicity included one or more of the followings: inactivity, collapse, hunched posture and tremors at the very high dose. Four males and all the females died by day 6, with the majority of deaths occurring by day 2 (very high). No drug related gross or microscopic changes were observed in animals (low or mid). Drug related pathologic changes were limited to the testis and spleen of the animals which were sacrificed at the

end to the postdose period (high and very high). In the testes, drug related microscopic lesions consisted of moderate degeneration of seminiferous tubular epithelium in three males (high) and the one surviving male (very high). In the spleen, drug related microscopic lesions consisted of mild to moderate lymphoid depletion (high, 3 males and 2 females; very high, 1 male). Doses of 1000 and 5000 mg/kg were overtly toxic and caused death. The acute maximum tolerated single oral gavage dose of entecavir in the mouse was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg or 973 mg/day (60 kg person).

2. Rats: Groups of male and female rats (5 animals/sex/group) received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high). Key findings: no drug related findings were noted at the low or mid dose. At the high dose, one rat died on day 6, and at very high dose, all rats died on days 3-7. Mild to marked body weight losses were noted in the rats that died. No clinical signs of toxicity were evident in the rat that died (high). Inactivity, chromodacryorrhea, scant feces, pale pinnae, paws and tail and/or fecal staining were seen in animals that died (very high). Drug related gross and microscopic lesions were seen only in the small intestine of animals that died. In these rats, there was red discoloration of the small intestine that was associated with moderate to marked hemorrhage and necrosis of the duodenum and jejunum. No drug related gross or microscopic lesions were seen in rats that survived to the end of the two week postdose period. Doses of 1000 and 5000 mg/kg were overtly toxic and caused death. The acute maximum tolerated single oral gavage dose of entecavir in the rat was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 32.46 mg/kg or 1.94 mg/day (60 kg person).

Repeat-dose toxicity

1. Two-week oral toxicity study in rats: Groups of male and female rats (10 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high) for a period of 2 weeks. In this study, entecavir was well tolerated at doses of 2 and 20 mg/kg/day. At 200 mg/kg/day, entecavir was overtly toxic, causing several deaths and lesion in a number of organs/tissue. The target organs included: gastrointestinal tract, heart, kidney, lymphoid organs, bone marrow and testes. Dose level of 2 mg/kg/day may be considered a NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.32 mg/kg/day (19.5 mg/day for a 60 kg person). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 0.7 μg*hr/ml in male and female rats.

2. Two week palatability study in rats: Groups of male and female rats were offered continuously in the diet entecavir at target mean daily dose of 0 (diet, controls), 30 (low), 100 (mid) or 300 (high) for two weeks. Based on adverse results, the high dose was lowered to 200 mg/kg/day on day 5 of dosing. Toxicokinetic evaluations on day 1 revealed that systemic exposures of entecavir increased with dose. No drug related changes were noted at the low dose. At the mid dose, decreases in body weights and food consumptions were noted. The high dose was overtly toxic and caused deaths of all

animals.

3. Two week palatability study in mice: Groups of male and female mice were offered entecavir continuously in the diet at target mean daily doses of 0 (drug free diet, controls), 25 (low), 100 (mid) or 400 mg/kg/day (high) for a period of two weeks. Based on adverse results obtained after 8 days of doing, the target dose for the high dose group was decreased to 200 mg/kg/day on day 10. No drug related changes were noted in mice at the low dose. At the mid dose, adverse effects on body weight gáin were evident. Administration of the high dose produced one death, body weight losses and adverse effect on food consumption. Toxicokinetic evaluations on day 17 revealed that systemic exposures to entecavir increased with dose.

4. Two-week oral toxicity in dogs: Groups of male and female beagle dogs received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 1 (low), 10 (mid) or 100 mg/kg/day (high) for 2 consecutive weeks. The high dose was lowered from 100 to 50 mg/kg starting on day 6. No drug related changes were seen at the low dose. The primary adverse finding at the mid dose was testicular degeneration. At the high dose, numerous adverse effects were observed including deaths, changes in a number of clinical pathology parameters and lesions in multiple organs/tissues. Many of the clinical pathology changes in dogs sacrificed in moribund condition were considered secondary to their debilitated state. Other changes in the high dose dogs such as decreased total leukocyte and platelet counts and increased plasma fibrinogen were consistent with the bone marrow depletion and inflammatory lesions, respectively. Degenerative changes were observed in the alimentary tract, bone marrow, lymphoid organs and testes. In this study, the NOEL was 1 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be 0.54 mg/kg/day (32.4 mg/day for a 60 kg person).). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 3.9 and 5.5 μg*hr/ml in male and female dogs, respectively.

5. One-month oral range finding toxicity in monkeys: Groups of male and female cynomolgus monkeys (2 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (1% sodium carboxymethylcellulose; vehicle control), 5 (low) or 25 mg/kg/day (high) for a period of one month. No drug related clinical signs or effect on body weights or food consumption were noted. No drug related changes in hematological or serum chemistry parameters were observed. No drug related gross lesions were seen. Drug related microscopic findings were limited to liver in both groups of monkeys and consisted of minimal to mild hepatocellular cytoplasmic vacuolation. In this study, a NOAEL could not be identified. Exposure (AUC_{ss}) was 0.655 and 2.24 μg*hr/ml at the low and high doses, respectively.

6. Three-month dietary toxicity study in rats: entecavir was administered in the diet to 6 groups of male and female rats at dose levels of 0 (diet only, controls), 1, 10, 40, 80 and 120 mg/kg/day for 3 months. At 40 mg/kg/day or more, numerous adverse effects were seen including deaths, changes in number of clinical pathology parameters and lesions in multiples organs and tissues. Increased serum AST and ALT were observed at all doses in this study. Degenerative changes were observed in the bone marrow, duodenum,

lymphoid organs and testes (organs/tissues containing rapidly dividing cells are potential targets). Other toxicological targets were kidneys, lungs, skeletal muscle and heart. Entecavir was well tolerated at a dose level of 1 mg/kg/day; this dose level may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.162 mg/kg/day (9.7 mg/day for a 60 kg person).). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 0.159 μg*hr/ml in male rats. The findings at 10 mg/kg/day suggested that this dose level is near the threshold dose of entecavir for substantive toxicity in rats. Doses of 40 mg/kg/day or more exceeded the MTD in rats as evidenced by deaths and lesions in multiple organs and tissues.

7. Three-month oral toxicity in dogs: Groups of male and female beagle dogs (3 animals/sex/group) received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) for 3 consecutive months. The high dose was lowered from 30 to 15 mg/kg starting on day 29. Inflammation of the central nervous system (CNS) was seen clearly in dose related manner. At the low dose, minimal inflammation in the brain occurred in one of six dogs. At the mid dose, minimal to mild brain inflammation occurred in two of six dogs and minimal spinal cord inflammation occurred in one of these dogs. At the high dose, three dogs that survived had mild to moderate brain inflammation and two of these dogs also had minimal to mild spinal cord inflammation. In these dogs, there were no overt CNS signs at any dose levels. No CNS changes were seen in a previous 2-week study in dogs. In 3-month rodent studies, histopathology results did not reveal any evidence of adverse effects on the CNS. The sponsor believed that the CNS inflammation may be a species specific finding in dogs. Rapidly dividing cells such as bone marrow and testes are the target organs for entecavir. In this study for CNS inflammation, a NOAEL could not be identified; however for other organs a dose level of 0.3 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be less than 0.05 mg/kg/day (2.9 mg/day for a 60 kg person). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 1.2 and 1.3 μg*hr/ml in male and female dogs, respectively.

8. Six-month oral range finding in mice: Groups of male and female CD-1 mice (20 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (sterile water; vehicle control), 0.2 (low), 1 (mid) or 5 mg/kg/day (high) for a period of 6 months. At the low dose, the liver was the only target organ, and the changes observed in the liver were minimal in severity. At the mid dose, changes in the liver were also minimal/ in addition, minimal changes in skeletal muscle and the lungs were seen. At the high dose, minimal to mild changes in the liver and minimal to mild changes in skeletal muscle and lungs were evident. The liver, skeletal muscle and lungs were the target organs in this study. A dose level of 5 mg/kg/day (high) may be considered the MTD for an oral carcinogenicity study. In this study, a NOAEL could not be determined; however, it should be less than 0.2 mg/kg/day. In this study at the low dose, the exposure was (AUC_{24ss}) 0.321 and 0.336 μg*hr/ml in male and female mice, respectively.

9. Six-month oral toxicity in rats: Groups of male and female rats (20 animals/sex/group) received once daily oral gavage doses of entecavir at dose levels of 0 (sterile water; vehicle control), 0.6 (low), 3 (mid) or 15 mg/kg/day (high) for a period of 6 months. The

target organs were liver and skeletal muscle at all doses tested. Degenerative changes in the liver associated with enlarged hepatocellular mitochondria and skeletal muscle myopathy were evident at all doses tested in this study. In a previous 3-month dietary study in rats, there were no drug related changes in the liver at the overtly toxic dose of 80 mg/kg/day and the threshold dose for skeletal muscle myopathy was 10 mg/kg/day. Thus, the toxicity of entecavir increased when the dosing period was extended beyond 3 months. In this study, a NOAEL could not be determined; however, it should be less than 0.6 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be less than 0.097 mg/kg/day (5.8 mg/day for a 60 kg person). In this study at the low dose, the exposure was (AUC_{24ss}) was 0.408 and 0.257 μg*hr/ml in male and female rats, respectively.

10. One-year oral toxicity study in monkeys: Groups of male and female cynomolgus monkeys (6 animals/sex/group) received once daily oral gavage doses of entecavir at dose levels of 0 (avicel/methocel/water preparation; vehicle control), 0.4 (low), 4 (mid) or 40 mg/kg/day (high). After 3 month of dosing, two males and two females from each group were sacrificed for an interim evaluation; the remaining monkeys were dosed for 12 months. No drug related changes were seen at the low or mid dose. At the high dose in males, minimally elevated mean serum urea nitrogen and potassium values were noted. Toxicokinetic evaluations indicated that there were dose related exposures to entecavir. No substantive differences in exposure to entecavir were evident between males and females. Mean CSF levels of entecavir were also dose related. In this study, no substantive toxic effects were seen in monkeys given up to 40 mg/kg/day for one year. A dose of 40 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 13.33 mg/kg/day (800 mg/day for a 60 kg person). In this study at the NOAEL, exposure (AUC_{24ss}) was 4.64 and 3.15 μg*hr/ml in male and female monkeys, respectively.

Genetic toxicology

1. Ames reverse mutation study in Salmonella and Escherichia coli: Entecavir was assessed for mutagenic potential in the Ames assay at concentrations up to 5000 μg/plate, both with or without S-9 metabolic activation. No cytotoxicity was observed in any of the tester strains at any concentration, either with or without metabolic activation. The histamine+ and tryptophan+ revertant frequencies in each of the treated cultures were essentially at the concurrent negative controls. Under the conditions of this study, entecavir was not mutagenic in the Ames reverse mutation assay.

2. In vitro transformation of Syrian hamster embryo cells: Primary cells from Syrian hamster embryos were exposed to entecavir for seven consecutive days at concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 μg/ml to evaluate morphologic transformations in the embryo. Also included in the study were the reference nucleosides acyclovir, ganciclovir and sorivudine, and a positive control, benzo(a)pyrene, a known rodent carcinogen. Results indicated no significant elevation in morphologically transformed colonies (MTCs) with entecavir. Of the three reference nucleosides, only sorivudine induced a statistically significant and toxicologically meaningful increase in MTCs. Under the

conditions of this study, entecavir was negative in the Syrian hamster embryo cell assay.

3. Cytogenetic study in primary human lymphocytes: Entecavir was tested for its potential to induce chromosome aberrations in cultured human lymphocytes with and without exogenous metabolic activation (rat liver homogenate from Aroclor-induced S-9 fraction) at dose levels of 2.5, 5, 10, 20, 50 or 200 μg/ml. Toxicity was assessed by measuring the reduction in the Relative Mitotic Index. The test article was tested with concurrent vehicle and positive controls. In this study, statistically significant elevations in the frequency of cells bearing metaphase chromosome aberration were seen at entecavir concentrations of 10 μg/ml or greater in the absence of S-9 enzymes and 50 μg/ml or greater in the presence of S-9 enzymes. Dose related and statistically significant decreases in mitotic index (cytotoxicity) were observed for cultures treated with entecavir in both the absence and presence of S-9 activation. Entecavir, at concentrations up to and including cytotoxic doses in both the absence and presence of S-9 metabolic activation, is clastogenic in the in vitro human lymphocyte chromosome aberration assay. Thus, it is concluded that entecavir is clastogenic to cultured human lymphocytes.

4. DNA repair study of entecavir in rats: Groups of male Sprague-Dawley rats (6/group) were orally gavaged with entecavir at single dose levels of 0 (control), 2 (low), 20 (mid), 200 (high) or 2000 mg/kg (very high). Three additional rats were given dimethylnitosamine orally at 10 mg/kg to serve as a positive control. Three treated animals and the positive control were euthanized at approximately 2 hr after dosing, and 3 treated animals and the vehicle controls were euthanized 16 hr after dosing to valuate the ability to induce unscheduled DNA synthesis (UDS). UDS evaluations for the treated rats, either 2 or 16 hr before sacrifice, were clearly negative, as were those for the vehicle control groups. Conclusions: under the conditions of this assay, entecavir did not induce genotoxic damage that resulted in UDS in male rats.

5. Oral micronucleus study in rats: Groups of male rats (5 animals/group) were orally gavaged with entecavir at dose levels of 0 (control), 2 (low), 20 (mid), 200 (high) or 2000 mg/kg/day (very high) for 3 days to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. Due to unexpected drug related deaths at 2000 mg/kg dose level, a portion of the study was repeated at dose levels of 200 and 1500 mg/kg. Bone marrow cells were collected 24 hr after the treatment and were examined for micronucleated polychromatic erythrocytes (PCEs). Two animals died (high) following the third dose. Drug related clinical signs were observed in the high dose animals. Mild bone-marrow toxicity was apparent in the surviving high dose animals. The frequencies of PCEs in the bone marrow of treated rats were not significantly increased ($p=0.05$) in the treated groups when compared to the controls. Under the conditions of the test and according to the criteria set for evaluating the test results, entecavir was negative for in vivo genotoxic potential in the rat micronucleus assay.

Carcinogenicity

1. oral (gavage) oncogenicity study in rats: The oncogenicity potential of entecavir was investigated in male rats at oral gavage dosages of 0.003 (low), 0.02 (mid), 0.2 (high) or

1.4 mg/kg/day (highest) and in females at dose levels of 0.01 (low), 0.06 (mid), 0.4 (high) or 2.6 mg/kg/day (highest) in comparison with vehicle controls for a period of 96 (male) and 104 (female) weeks. The NOEL for neoplasia was 0.2 mg/kg/day for males and 0.06 mg/kg/day for females. At tumorigenic doses, systemic exposures were 35- and 4-times that in humans (1.0 mg daily dose) in male and female rats, respectively. The treatment induced hepatocellular adenomas in female rats were significant (p=0.005) at the highest dose level. Combined adenomas and carcinomas in the female rats were also significant (p=0.005) at the highest dose. In female rats, the combined incidence of adenomas and carcinomas was 1% (controls), 4% (low), 5% (mid), 2% (high) and 18% (highest). The exposure in the female rats (highest) was 24 times the exposure in humans. Brain gliomas were significant (p=0.025) at the highest dose in both male and female rats. In male rats, the incidence was 0% (controls), 2% (low), 2% (mid), 3% (high) and 7% (highest). In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 0% (high) and 5% (highest). The exposures in the male and female rats (highest) were 35 and 24 times the exposure in humans, respectively. The skin fibromas in female rats were significant (p=0.025) at the high and highest doses. In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 3% (high) and 5% (highest). The exposures in the female rats were 4 (high) and 24 (highest) times the exposure in humans. With regard to non-neoplastic changes, hyperplastic changes were noted in the pancreas for males (high and highest). In addition, non proliferative findings were present in the liver for males and females (high and highest) and the testes and kidneys for males (highest). The NOELs for non-neoplastic changes were 0.02 mg/kg/day for males and 0.06 mg/kg/day for females.

2. Oral (gavage) oncogenicity study in mice: The oncogenicity potential of entecavir was investigated in mice at oral gavage dosages of 0.004 (low), 0.04 (mid), 0.4 (high) or 4.0 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The NOEL for neoplasia was 0.004 mg/kg/day for males, based on pulmonary adenomas; for all other tumors in males and females, the NOEL was 0.4 mg/kg/day. At the tumorigenic dose in male mice, systemic exposure was 3-times that in humans (1.0 mg daily dose). The treatment induced bronchioloalveolar adenomas in male mice achieved statistical significance (p=0.005; mid, high and highest) and increased in a dose related manner at exposures multiples of 3 and higher relative to the daily clinical dose of 1.0 mg. In male mice, combined lung adenomas and carcinomas also achieved statistical significance (p=0.005) increased in the dose related manner at exposures multiples of 3 and higher relative to the daily clinical dose of 1.0 mg. In male mice, the combined incidence of adenomas and carcinomas was 12% (controls), 20% (low), 26% (mid), 40% (high) and 58% (highest). In female mice, the combined incidence of adenomas and carcinomas was 20% (controls), 13% (low), 10% (mid), 35% (high) and 52% (highest). Hepatocellular carcinomas in the male mice were significant (p=0.005) at the highest dose level. Combined liver adenomas and carcinomas were also significant (p=0.005) at the highest dose level in the male mice. In male mice, the combined incidence of adenomas and carcinomas was 11% (controls), 9% (low), 8% (mid), 16% (high) and 25% (highest). The exposure in the male mice was 42 times the exposure in humans. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant (p=0.005) at the highest dose level. In female mice, the incidence of all vascular tumors was 16% (controls), 23% (low), 29% (mid), 26% (high)

and 64% (highest). The exposure in the female mice was 40 times (highest) the exposure in humans. Non-neoplastic changes noted after the administration of entecavir to mice included inflammatory changes in the lungs for males and females (high and highest) and ovarian lesions for females (highest). The NOEL for non-neoplastic changes was 0.04 mg/kg/day for males and females (3-time the exposure in humans).

Reproductive and developmental toxicology

1. Oral study of fertility and early embryonic development in female rats: Female rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) once daily for approximately 2 weeks. There were no drug related changes at any dose tested. Entecavir had no effect on reproductive function in females (estrous cycling, mating and fertility) or on the early embryonic development of the offsprings at any dose level. In this study, a dose level of 30 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent oral dose in humans would be 4.87 mg/kg/day (292 mg/day for a 60 kg person).

2. Oral study of fertility and early embryonic development in male rats: Male rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.1 (low), 1 (mid) or 10 mg/kg/day (high) once daily for approximately 4 weeks. Entecavir had no effect on fertility, mating or early embryonic development of the offsprings at any dose level. Similarly, reproductive organs weights (testes, epididymides and prostate/seminal vesicles) and sperm parameters (motility, morphology and counts) were unaffected by the treatment. In this study, a dose level of 1 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.16 mg/kg/day (9.7 mg/day for a 60 kg person). Entecavir had no effect on reproductive function in male rats. A dose level of 10 mg/kg/day may be considered the NOEL for male reproductive toxicity. Based on the body surface area factor, an equivalent oral dose in humans would be 1.6 mg/kg/day (97.4 mg/day for a 60 kg person).

3. Oral study of embryo-fetal development in rats: Presumed pregnant female rats received oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high) in this study, entecavir caused both maternal and embryo fetal toxicity at mid and high doses. Fetal malformation of the tail and vertebrae and developmental delays in ossification of the vertebrae, sternebrae and phalanges and increases in the number of lumbar vertebrae were seen at the high dose. In this study, a dose level of 2 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.32 mg/kg/day (19.5 mg/day for a 60 kg person).

4. Oral study of embryo-fetal development in rabbits: Presume pregnant female rabbits received repeated oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 1 (low), 4 (mid) or 16 mg/kg/day (high). In this study, statistically significant increases in the average number of ossified ribs (13^{th} rib) occurred at all doses

tested.

Since statistically significant increases in the average number of ossified ribs (13th rib) occurred at all doses tested in this study.

No drug related maternal changes were seen at any dose. Drug related increased in embryo-fetal death (resorptions) with associated decreases in liver-litter sizes occurred at the high dose. Approximately half of all conceptuses of does (high) were resorbed. Entecavir caused embryo-fetal toxicity (skeletal variant) at the low, mid or high dose without producing maternal toxicity.

5. One-week toxicokinetics study in pregnant rabbits: Two groups of presumed pregnant rabbits received repeated oral gavage doses of entecavir at dose levels of 4 (mid) or 16 mg/kg/day (high) once daily on days 6 through 12 of gestation. On day 13 of gestation, the rabbits were sacrificed and necropsied, and pregnancy statuses were confirmed. Plasma concentrations were determined for all pregnant rabbits. Cmax was achieved at a median Tmax value of 0.5 hr. Mean Cmax and AUC24 hr values were 1.7 and 7.3 μg/ml and 5.6 and 23.4 μg*hr/ml at mid and high doses, respectively. Overall, the results indicated that there were dose related systemic exposures of pregnant rabbits to entecavir during the one week toxicokinetic study.

6. Oral study of pre- and postnatal development in rats: Groups of presumed pregnant rats (25/group) received entecavir via oral gavage at dose levels of 0 (vehicle controls), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) from day 6 of gestation through day 20 of lactation. At the high dose level, a transient and mild reduction in maternal body weight gain occurred during gestation. There were no drug related findings in the F1 generation offspring at any dose level. Entecavir caused drug related changes in the F0 dams at the high dose level, without affecting the pre- or postnatal development of the F1 generation offspring at any dose level. A dose level of 3 mg/kg/day may be considered the NOEL for F0 dams. Based on the body surface area factor, an equivalent oral dose in humans would be 0.48 mg/kg/day (29.2 mg/day for a 60 kg person). A dose level of 30 mg/kg/day may be considered the NOEL for F1 offsprings. Based on the body surface area factor, an equivalent oral dose in humans would be 4.87 mg/kg/day (292 mg/day for a 60 kg person).

2.6.6.2 Single-dose toxicity

Study title: 1. Entecavir: Acute toxicity in mice after oral administration

Key study findings: Groups of male and female mice (5 animals/sex/group) received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high). Key findings: beginning day 3, dose-related, transient, minimal to marked body weight losses were noted (mid, high and very high). Dyspnea, collapse and death were noted in one male mouse (high). Overt signs of toxicity included one or more of the following: inactivity, collapse, hunched posture and tremors at the very high dose. Four males and

all the females died by day 6, with the majority of deaths occurring by day 2 (very high). No drug related gross or microscopic changes were observed in animals (low or mid). Drug related pathologic changes were limited to the testis and spleen of the animals which were sacrificed at the end to the postdose period (high and very high). In the testes, drug related microscopic lesions consisted of moderate degeneration of seminiferous tubular epithelium in three males (high) and the one surviving male (very high). In the spleen, drug related microscopic lesions consisted of mild to moderate lymphoid depletion (high, 3 males and 2 females; very high, 1 male).

Doses of 1000 and 5000 mg/kg were overtly toxic and caused death. The acute maximum tolerated single oral gavage dose of entecavir in the mouse was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg or 973 mg/day (60 kg person).

Study no.: 96026

Volume # and page #: 1 and page # 1-37

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: November 8, 1996

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # CO11A, → pure

Methods

Doses: animals received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high).

Species/strain: male and female mice, strain: → (CD-1)

Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, volume: 1-40 ml/kg

Age: one week after arrival

Weight: 18-25 g

Mortality and clinical signs: the animals were examined daily for changes in condition and behavior. Observation of moribund or dead animals was made twice daily.

Gross pathology: animals found dead were necropsied as soon as possible. All other mice were sacrificed with carbon dioxide after 2-week postdose observation. The following organs and tissues were examined: gross lesions, adrenal glands, cervix, epididymides, eyes, gallbladder, heart, kidneys, large intestine, liver, lung, lymph node, mammary gland, ovaries, pancreas, prostate gland, seminal vesicle, skin, small intestine, spleen, stomach, testes, thymus, urinary bladder, uterus and vagina.

Histopathology: Adequate Battery: yes, Peer review: no

Results

Mortality: No death occurred at the low or mid dose. High: one male died on day 2; very high: four males died, three on day 2 and one on day 4. All females died at the very high dose level, three on day 2, one on day 3 and one on day 6.

Clinical signs: no drug related signs overt toxicity were noted at the low or mid dose. At the high dose, one male had dyspnea and collapsed prior to death on day 2. At the very high dose, three males and three females collapsed on day 2, and one male and one female were inactive on day 2. In addition, the female showed hunched posture and tremors on day 3.

Body weights: no drug related changes in body weights were seen at the low or mid dose. At the high dose, mean body weights at the end of the observation period were 13% (males) and 6% (females) lower than the controls and transient minimal to marked body weight losses were noted in most of the surviving mice during the study, beginning on day 3. The one surviving male (very high) showed marked body weight loss during the study, but recovered somewhat by the end of the observation period.

Gross pathology and histopathology: no drug related gross lesions were seen in any of the mice that died. In the testes, drug related microscopic lesions consisted of bilateral moderate multifocal degeneration of seminiferous tubular epithelium in 3 males (high) and in the one male (very high) that survived to the scheduled necropsy. The degeneration of seminiferous tubules correlated grossly with slightly decreased size of the testes of the four males. The degeneration in the seminiferous tubules was characterized by reduced numbers of tubular epithelium resulting in a decrease in the diameter of affected tubules when compared to the controls. In the spleen, drug related microscopic lesions consisted of mild to moderate diffuse lymphoid depletion in three males and two females (high) and in the one male (very high) that survived to the scheduled necropsy. The lymphoid depletion in the spleen correlated grossly with slightly decreased size of the spleen in two males (high) and in the one male (very high).

Study title: 2. Entecavir: single dose oral toxicokinetics study in mice

Key study findings: The single dose toxicokinetics of entecavir after oral gavage (2.5, 10 or 40 mg/kg) administrations was investigated in male CD-1 mice (15/group). Serial blood samples were obtained after the dosing and were analyzed by a validated HPLC

method. Key findings: are shown in Table 19. Dose related, but not dose proportional increases in exposure (AUC or Cmax) of mice to entecavir were apparent over the dose range of 2.5 to 40 mg/kg.

Study no.: 97030

Volume # and page #: 1 and page # 1-36

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: December 1, 1997

GLP compliance: no

QA report: no

Methods

Doses and methods: The single dose pharmacokinetics of entecavir after oral gavage (2.5, 10 or 40 mg/kg) administrations was investigated in male CD-1 mice (15/group). Serial blood samples were obtained at 0.5, 1, 4, 8 and 24 hr after the dosing and were analyzed by a validated HPLC method.

Species/strain: mice, strain· →. . (CD-1)

Results

Toxicokinetics: mean toxicokinetic parameters are shown in Table 1. There was a dose related, but not dose proportional increase in exposures (AUC or Cmax) of mice to entecavir po administration (doses ranging from 2.5 to 40 mg/kg).

Table 19

Single dose pharmacokinetics of entecavir in male mice after po administration.

Dose (mg/kg)	Tmax (hr)	Cmax (µg/ml)	AUC0-24 (µg*hr/ml)
2.5	1	0.75	1.97
10	0.5	1.3	2.63
40	0.5	9.1	15.61

Study title: 3. Entecavir: Single dose oral toxicity study in rats

Key study findings: Groups of male and female rats (5 animals/sex/group) received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel;

vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high). Key findings: no drug related findings were noted at the low or mid dose. At the high dose, one rat died on day 6, and at very high dose, all rats died on days 3-7. Mild to marked body weight losses were noted in the rats that died. No clinical signs of toxicity were evident in the rat that died (high). Inactivity, chromodacryorrhea, scant feces, pale pinnae, paws and tail and/or fecal staining were seen in animals that died (very high). Drug related gross and microscopic lesions were seen only in the small intestine of animals that died. In these rats, there was red discoloration of the small intestine that was associated with moderate to marked hemorrhage and necrosis of the duodenum and jejunum. No drug related gross or microscopic lesions were seen in rats that survived to the end of the two week postdose period.

Doses of 1000 and 5000 mg/kg were overtly toxic and caused death. The acute maximum tolerated single oral gavage dose of entecavir in the rat was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 32.46 mg/kg or 1.94 mg/day (60 kg person).

Study no.: 96027

Volume # and page #: 1 and page # 1-37

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: November 11, 1996

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # CO11A. — pure

Methods
Doses: animals received a single oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 40 (low), 200 (mid), 1000 (high) or 5000 mg/kg (very high).

Species/strain: male and female rats, strain: — Sprague-Dawley outbred albino rats

Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, volume: 1-125 ml/kg

Age: one week after arrival

Weight: 175-185 g

Mortality and clinical signs: the animals were examined daily for changes in condition and behavior. Observation of moribund or dead animals was made twice daily.

Gross pathology: animals found dead were necropsied as soon as possible. All other rats were sacrificed with carbon dioxide after 2-week postdose observation. The following organs and tissues were examined: gross lesions, adrenal glands, cervix, epididymides, eyes, heart, kidneys, large intestine, liver, lung, lymph node, mammary gland, ovaries, pancreas, prostate gland, seminal vesicle, skin, small intestine, spleen, stomach, testes, thymus, urinary bladder, uterus and vagina.

Histopathology: Adequate Battery: yes, Peer review: no

Results

Mortality: No death occurred at the low or mid dose. One rat died on day 6 (mid) and all rats died at the very high dose; two on day 3 and one each at days 4, 5 and 7.

Clinical signs: no drug related signs overt toxicity were noted at the low, mid or high dose. At the high dose, one male had dyspnea and collapsed prior to death on day 2. At the very high dose, two rats had chromodacryorrhea, scant feces and pale pinnae, paws, and tails. One of these rats was inactive and had fecal staining on days 4 and 5.

Body weights: no drug related changes in body weights were seen at the low or mid dose. At the high dose, mean body weights at the end of the observation period were 4% to 6.9% lower than the controls with marked body weight losses noted on days 3 and 5 in rats that died. At the very high dose, mild to marked body weight losses were evident on days 3 and 5.

Gross pathology and histopathology: drug related gross and microscopic lesions were observed only in the small intestine of the rats that died. The drug related lesions in these rats consisted of red discoloration in the small intestine that was associated with moderate to marked hemorrhage and necrosis of the mucosa in the duodenum and jejunum. In the duodenum and jejunum, there was extensive necrosis of crypt and villus epithelium and hemorrhage in the lamina propria, submucosa, and/or smooth muscle layers. In one rat (high that died), the small intestine had red discoloration. At necropsy, all of the rats that died had red discoloration of the lung as a result of moderate congestion seen microscopically. Since there were no degenerative changes associated with the congestion, this change was most likely related to the poor condition and death of these animals. No drug related gross or microscopic lesions were observed in rats examined at the end of the 2-week postdose observation period.

One rat (control), three rats (mid) and two rats (high) had mild to moderate multifocal subacute inflammation in the lung.

2.6.6.3 Repeat-dose toxicity

Study title: 1. Entecavir: 2-week oral toxicity study in rats

Key study findings: Groups of male and female rats (10 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high) for a period of 2 weeks. In this study, entecavir was well tolerated at doses of 2 and 20 mg/kg/day. At 200 mg/kg/day, entecavir was overtly toxic, causing several deaths and lesion in a number of organs/tissue. The target organs included: gastrointestinal tract, heart, kidney, lymphoid organs, bone marrow and testes. Dose level of 2 mg/kg/day may be considered a NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.32 mg/kg/day (19.5 mg/day for a 60 kg person). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 0.7 μg*hr/ml in male and female rats.

Study no.: 96028

Volume # and page #: 1 and pages # 1-417

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: April 22, 1997

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # CO11A,

Methods

Doses: animals received once daily oral gavage dose of entecavir at dose levels of 0 (1% avicel/0.25% methocel; vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high) for a period of 2 weeks.
Species/strain: rats — Sprague Dawley outbred

Number/sex/group or time point (main study): 10 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: Plasma concentrations of entecavir were determined at 0.5, 1, 2, 4, 8 and 24 hr after the first and 14th doses of the study.

Age: 5 weeks old

Weight: 105 to 121 g for males and 95 to 114 g for females

Sampling times: blood samples for clinical pathology were taken on days 9-11.

Results

Mortality: The high dose was overtly toxic, resulting in the death of 4 males and 2 females (days 13-16). There were no deaths at the low or mid dose.

Clinical signs: drug related in-life changes were limited to an increased incidence of poikilocytosis in females (low); this change was also evident in males (mid or high). There were no other drug related overt signs noted at the low or mid dose.

Body weights: no drug related body weight changes were noted at the low or mid dose. At the high dose, decreased body weight gains (body weights approximately 30% lower in males and 8% lower in females compared to the controls) were noted.

Food consumption: No drug related changes in food consumption (measured once weekly over a 24 hr period) were noted at the low or mid dose. At the high dose, mild to marked (most males) and moderate or marked (some females) decreases were observed.

Hematology: drug related hematologic changes at the low dose were limited to an increased incidence of poikilocytosis in females; this change was also evident in females at the mid dose and in males and females (high). **Mid dose:** other findings included mild increases in hemoglobin, hematocrit and erythrocyte count, and minimal decreases in mean corpuscular hemoglobin concentration in males (these changes were also present at the high dose); and in one female, a moderate decrease in bone marrow myeloid/erythroid ratio. **High dose:** a moderate decrease in leukocyte count and mild or marked decreases in platelet count in male rats that died, markedly decreased eosinophil counts, a mild increase in plasma fibrinogen (female), minimal to mild increases in prothrombin time, and a moderate decrease in bone marrow myeloid/erythroid ratio.

Clinical chemistry: there were no drug related changes at the low or mid dose.
High dose: mild to moderate increases in serum ALT and AST and mild to moderate deceases in serum potassium and mild decreases in serum triglycerides (males), albumin and phosphorus (males) were observed.

Urinalysis: there were no drug related changes at the low or mid dose. High: mild to moderate increases in urine output, with accompanying decreases in urine specific gravity were seen.

Gross pathology: Unscheduled-necropsied animals: drug related gross lesions were found in the dead animals and included: red luminal contents and/or moderate to marked red discoloration of the duodenal mucosa in 3 males and one female; mild to moderate decreased size of the thymus and grayish-green discoloration of the right testis in one male.

End of dose sacrificed animals: no drug related pathologic changes were observed at the low or mid dose. High dose: decreased thymus, spleen (males), prostate and uterus weights occurred. Gross lesions included red mucosal discoloration and red lumenal contents of the duodenum; red discoloration of the stomach mucosa; pale discoloration of the kidney and myocardium of the heart; and a decrease in the size of the thymus.

Organ weights drug related changes occurred only at the high dose and consisted of decreased thymus, spleen, prostrate and uterus weights as compared to the controls.

Histopathology: Adequate Battery: yes; Peer review: yes

Histopathology: no drug related changes were seen at the low dose. At the mid dose, the only drug related change was myeloid depletion of the bone marrow in one female rat. At the high dose, drug related microscopic lesions were observed in several organs and tissues of all animals. These lesions were similar in unscheduled-necropsied and end-of-dose sacrificed animals. At the high dose, changes included transmural hemorrhage, necrosis of villi and fibrin micro-thrombosis of the duodenum; tubular nephrosis of the kidney; coagulative necrosis of the myocardium and endocardial thrombosis of the heart; lymphoid atrophy of the thymus; myeloid depletion and sinusoidal congestion in the bone marrow; lymphoid necrosis and multinucleated giant cell infiltration in the lymph nodes; splenic lymphoid necrosis; and seminiferous tubular degeneration of the testes. Ultrastructural evaluation of livers (high) did not reveal any adverse findings.

Toxicokinetics: summary of mean toxicokinetic parameters is shown in Table 20. Toxicokinetic evaluations on days 1 and 14 revealed that systemic exposures to drug increased with dose; however, the increases generally were less than dose proportional. Peak plasma levels were achieved in 1-2 hr.

Table 20
Summary of mean toxicokinetic parameters of entecavir in rats

Group; dosage (mg/kg/day)		AUC_{24} (µg*hr/ml)		Cmax (µg/ml)	
		drug days		drug days	
		1	14	1	14
low 2	male	1.8	0.7	0.4	0.2
	female	0.6	0.7	0.28	0.16
mid 20	male	11.6	9.5	2.6	2.4
	female	5.5	4.8	1.8	1.2
High 200	male	58.6	60	11.9	7.6
	female	50.6	82.3	8.3	9.4

Study title: 2. Entecavir: two-week oral toxicity in dogs

Key study findings: Groups of male and female beagle dogs received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 1 (low), 10 (mid) or 100 mg/kg/day (high) for 2 consecutive weeks. The high dose was lowered from 100 to 50 mg/kg starting on day 6. No drug related changes were seen at the low dose. The primary adverse finding at the mid dose was testicular degeneration. At the high dose, numerous adverse effects were observed including deaths, changes in a number of clinical pathology parameters and lesions in multiple organs/tissues. Many of the clinical pathology changes in dogs sacrificed in moribund condition were considered secondary to their debilitated state. Other changes in the high dose dogs such as decreased total leukocyte and platelet counts and increased plasma fibrinogen were consistent with the bone marrow depletion and inflammatory lesions, respectively. Degenerative changes were observed in the alimentary tract, bone marrow, lymphoid organs and testes. In this study, the NOEL was 1 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be 0.54 mg/kg/day (32.4 mg/day for a 60 kg person).). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 3.9 and 5.5 µg*hr/ml in male and female dogs, respectively.

Study no.: Study No. 96029

Volume #, and page #: 1 and 1-364

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: April 8, 1997

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # CO13A. –

Methods

Doses: dogs received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 1 (low), 10 (mid) or 100 mg/kg/day (high) for 2 consecutive weeks. The high dose was lowered from 100 to 50 mg/kg starting on day 6.

Species/strain: beagle dogs

Number/sex/group or time point (main study): 3 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage

Age: 6-11 months old

Weight: 9.1-12.4 kg

Sampling times: Blood samples for toxicokinetic investigations were collected at 0.5, 1, 2, 4, 6, 8 and 24 hr post dosing on days 1 and 9. Plasma concentrations of entecavir were determined by a validated LC/MS method.

Results

Mortality: High dose: one male and one female dog were found dead on day 5 and 2 females were sacrificed in moribund condition on days 6 and 10. No death occurred at the low or mid dose.

Clinical signs: no drug related in-life changes were noted in animals (low or mid). The female (high) sacrificed on day 6 was inactive and had loose dark feces, abdominal discomfort and tenseness, dehydration, mild conjunctivitis and injected sclera. The female sacrificed on day 10 was thin, very weak, depressed and had dark tar-like feces, pale mucous membranes, harsh lung sounds, increased rectal temperature and a weak pulse. In the surviving dogs, an increased incidence of emesis (1-2 hr postdose) was seen; emesis was also observed in dogs that died or were sacrificed.

Body weights: no body weight changes were seen at the low or mid dose. At the high dose, mild to moderate decreases in body weight (16 to 24%) were seen.

Food consumption: no food consumption changes were seen at the low or mid dose; at the high dose, decrease in food consumption was approximately (30-50%).

Hematology: there were no drug related changes at the low dose. Mid dose: mild decreases in total leukocyte count and platelet counts in one male and one female were observed. At the high dose, changes included marked decreases in reticulocytes, total leukocyte and platelet counts; an increased incidence of poikilocytosis; and a marked increase in plasma fibrinogen. In the bone marrow, there was mostly marked depletion of all cell lines.

Clinical chemistry: there were no drug related changes at the low or mid dose. At the high dose in surviving animals, a mild increase in serum cholesterol was seen in one male. In the two females sacrificed in moribund condition, numerous changes were observed. The changes included marked increases in BUN, creatinine, glucose and phosphorus; and marked decreases in serum sodium and chloride. Moderate increases in serum ALP, bilirubin, cholesterol, total protein, phosphorus; and moderate decreases in serum sodium, potassium, and chloride were seen.

Urinalysis: no changes were seen at the low or mid dose. At the high dose, the only change was occult blood in the urine in one male.

EKG: there were no drug related changes observed in any dog.

Organ weights no drug related pathologic changes were observed at the low dose. The

only drug related organ weight change was decreased testes weights in male animals (mid and high).

Gross pathology:

Unscheduled necropsied animals: mild to marked red discoloration was observed grossly in various organs and tissues, including: mucosa of stomach (3 dogs), esophagus (one dog), large intestine (2 dogs), esophagus, and/or small intestine; liver; cervical lymph node; and/or spleen. The red discoloration was generally attributable to congestion and/or hemorrhage associated with the death or poor clinical condition of these animals.

End of dose sacrificed animals: no drug related gross lesions were seen in dogs (low or mid). At the high dose, lesions consisted of minimal to mild, focal or multifocal red discoloration in several areas of the alimentary tract including the mucosa of the small and large intestines, mesenteric lymph node and tongue.

Histopathology: Adequate Battery: yes; peer review: yes

In unscheduled necropsied animals (one male and three females), drug related lesions were observed in the following organs and tissue: bone marrow, small and large intestines, thymus, lymph nodes, spleen, kidneys and testes. In bone marrow, mostly marked depletion of all cell lines, resulting in generalized bone marrow hypocellularity, was seen in all unscheduled necropsied dogs. In the small intestine, microscopic lesions consisted of minimal to moderate mucosal-epithelial necrosis in all dogs/ mild lymphoid depletion and reticuloendothelial (RE) cell hypertrophy/hyperplasia in lymphoid nodules in the male and female; and mild mucosal hemorrhage in females, which correlated with the red discoloration observed grossly. In the large intestine, mild multifocal mucosal epithelial necrosis was seen in one female. In the thymus, moderate to marked diffuse atrophy was evident in all animals. In the lymph nodes, microscopic lesions consisted of mild to moderate RE cell hypertrophy/hyperplasia and necrosis in male and female animals. In spleen, RE cell hypertrophy/hyperplasia was observed in all animals. In the kidneys, microscopic lesions consisted of mild to moderate tubular nephrosis in all animals; mild pelvic epithelial degeneration in male and female animals and minimal thrombosis in both male and female animals. In testes, mild multifocal seminiferous tubular degeneration was seen in all males.

End of dose sacrificed animals: no drug related lesions were seen at the low dose. Mid dose: the only drug related microscopic lesion was minimal to mild seminiferous tubular degeneration in the testes. High dose: drug related microscopic lesions included: depletion of all cell lines in the bone marrow; mucosal epithelial necrosis, lymphoid depletion, reticuloendothelial cell hypertrophy/hyperplasia, acute inflammation, and hemorrhage in the intestines; atrophy of the thymus; reticuloendothelial cell hypertrophy/hyperplasia in the lymph nodes and spleen; tubular nephrosis, pelvic epithelial degeneration and thrombosis in the kidneys; and seminiferous tubular degeneration in the testes.

Toxicokinetics: mean toxicokinetic parameters are shown in Table 21. Evaluations on days 1 and 9 revealed that there were dose related exposures to drug. On day 1, AUC values were greater than dose proportional Exposures were higher on day 9 than on day 1 (low and mid) dose animals. Peak plasma concentrations were achieved in 1-6 hr.

Table 21
Summary of mean toxicokinetic parameters of entecavir in dogs

Group; dosage (mg/kg/day)		AUC_{24} (µg*hr/ml)		Cmax (µg/ml)	
		drug days		drug days	
		1	9	1	9
low 1	male	2.8	3.9	0.8	0.9
	female	3.3	5.5	0.9	1.1
mid 10	male	50.8	105.9	8	10.8
	female	52.1	54.7	9.1	12.1
High 100/50	male	886.2	224.4	72.7	28.6
	female	562.3	300.9	46.8	48

Study title: 3. Entecavir: two week palatability study in mice

Key study findings: groups of male and female mice were offered entecavir continuously in the diet at target mean daily doses of 0 (drug free diet, controls), 25 (low), 100 (mid) or 400 mg/kg/day (high) for a period of two weeks. Based on adverse results obtained after 8 days of dosing, the target dose for the high dose group was decreased to 200 mg/kg/day on day 10. No drug related changes were noted in mice at the low dose. At the mid dose, adverse effects on body weight gain were evident. Administration of the high dose produced one death, body weight losses and adverse effect on food consumption. Toxicokinetic evaluations on day 17 revealed that systemic exposures to entecavir increased with dose.

Study no.: 96044

Volume # and page #: 1 and 1-56

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: April 1, 1997

GLP compliance: no

QA report: no

Drug, lot #, and % purity: CO11A, CO12A and CO13A; [redacted]

Methods

Doses: of 0 (drug free diet, controls), 25 (low), 100 (mid) or 400/200mg/kg/kday (high)

Species/strain: outbred albino mice

Number/sex/group or time point (main study): 9/sex/goup

Route, formulation, volume, and infusion rate: orally in diet

Satellite groups used for toxicokinetics: 9 mice/sex/group

Age: 5 weeks

Weight: 24.2 to 28.6 g for males and 20.2 to 23.3 g for females

Results

Mortality: one female (high) was found dead on day 16 of dosing

Clinical signs: no overt signs were noted

Body weights: no drug related changes were noted at the low dose. At the mid dose, minimal weight losses and minimal to mild decreased in body weight gain were evident, mostly during the second week of the study. At the high dose, body weight gains were evident by day 8. Weight losses were no longer evident by day 12 following reduction of the target dose from 400 to 200 mg/kg; however, suppression of weight gain was still present.

Food consumption: no drug related changes in food consumption were noted in mice (low or mid). Sporadic mild to moderate decreases in food consumption were noted animals at the high dose.

Entecavir intake: is shown in Table 22

Table 22
Approximate average entecavir intake in the diet

Group	Target dose (mg/kg/day)	Average intake (mg/kg/day)	
		Male	Female
Low	25	26	27
Mid	100	113	93
high	400/200	332	346

Toxicokinetics: mean plasma concentrations are shown in Table 23. Toxicokinetic

evaluations on day 17 revealed that systemic exposures to entecavir increased with an increase in dose.

Table 23
Mean plasma concentration time data in mice on day 17

Dose (mg/kg/day)	Time (hr)	Mean plasma concentration (μg/ml)	
		male	female
25	4	0.245	0.351
	8	0.643	0.420
	24	0.442	0.99
100	4	1.47	0.889
	8	0.935	1.96
	24	1.17	0.817
400/200	4	3.0	2.21
	8	2.96	3.94
	24	1.22	0.725

Study title: 4. Entecavir: two week palatability study in rats

Key study findings: Groups of male and female rats were offered continuously in the diet entecavir at target mean daily dose of 0 (diet, controls), 30 (low), 100 (mid) or 300 (high) for two weeks. Based on adverse results, the high dose was lowered to 200 mg/kg/day on day 5 of dosing. Toxicokinetic evaluations on day 1 revealed that systemic exposures of entecavir increased with dose. No drug related changes were noted at the low dose. At the mid dose, decreases in body weights and food consumptions were noted. The high dose was overtly toxic and caused deaths of all animals.

Study no.: 96045

Volume # and page #: 1 and 1-61

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: April 1, 1997

GLP compliance: no

QA report: no

Drug, lot #, and % purity: CO12A, —

Methods

Doses: 0 (diet, controls), 30 (low), 100 (mid) or 300/200 mg/kg/day (high)

Species/strain: rats, — Sprague-Dawley outbred albino

Number/sex/group or time point (main study): 6 animals/sex/group

Route, formulation, volume, and infusion rate: orally, diet

Satellite groups used for toxicokinetics: only for toxicokinetics

Age: 5 weeks

Weight: 115-129 g for males and 102-115 g for females

Sampling times: plasma samples were obtained at 4, 8 and 24 hr on day 14 of the study

Results

Mortality: in the high dose all animals were found dead between day 6 and 12 of dosing

Clinical signs: no overt signs of toxicity were seen in rats, even those died.

Body weights: no drug related change was seen at the low dose. In males at the mid dose, mild to moderately lower body weights compared to the controls were seen during the last few days of dosing. In females (mid), a minimal adverse effect on body weight was observed. In the high dose group, marked adverse effects on body weights were seen; weight losses were evident by day 3 of the study.

Food consumption: no substantive changes were noted in rats at the low dose. Moderate decreases in food consumption were consistently noted in males (mid); sporadic mild decreases were noted in females at this dose level. Marked decreases in food consumption were noted in the high dose animals.

Entecavir intake: is shown in Table 24

Table 24
Approximate average entecavir intake in the diet

Group	Target dose (mg/kg/day)	Average intake (mg/kg/day)	
		Male	Female
Low	30	30	28
Mid	100	93	98
high	300/200	138	138

Toxicokinetics: due to toxicity, all animals in the high dose group died before blood could be taken for toxicokinetic evaluation. Mean plasma concentrations are shown in Table 25. Toxicokinetic evaluations on day 17 revealed that systemic exposures to entecavir increased with an increase in dose.

Table 25
Mean plasma concentration time data in rats on day 14

Dose (mg/kg/day)	Time (hr)	Mean plasma concentration (μg/ml)	
		Male	female
30	4	0.844	0.487
	8	0.973	0.635
	24	0.408	0.614
100	4	4.59	0.80
	8	3.83	1.30
	24	2.64	1.54

Study title: 5. Entecavir: three month dietary toxicity study in rats

Key study findings: entecavir was administered in the diet to 6 groups of male and female rats at dose levels of 0 (diet only, controls), 1, 10, 40, 80 and 120 mg/kg/day for 3 months. At 40 mg/kg/day or more, numerous adverse effects were seen including deaths, changes in number of clinical pathology parameters and lesions in multiples organs and tissues. Increased serum AST and ALT were observed at all doses in this study. Degenerative changes were observed in the bone marrow, duodenum, lymphoid organs and testes (organs/tissues containing rapidly dividing cells are potential targets). Other toxicological targets were kidneys, lungs, skeletal muscle and heart. Entecavir was well tolerated at a dose level of 1 mg/kg/day; this dose level may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.162 mg/kg/day (9.7 mg/day for a 60 kg person).). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 0.159 μg*hr/ml in male rats. The findings at 10 mg/kg/day suggested that this dose level is near the threshold dose of entecavir for substantive toxicity in rats. Doses of 40 mg/kg/day or more exceeded the MTD in rats as evidenced by deaths and lesions in multiple organs and tissues.

Study no.: 97001

Volume # and page #: 1 and 1-564

Conducting laboratory and location: `Bristol-Myers Squibb, New Brunswick, NJ`

Date of study completion: April 23, 1998

GLP compliance: yes

QA report: yes; Peer review: yes

Drug, lot #, and % purity: R022A and [redacted]

Methods

Doses: are listed in Table 26.

Table 26
Experimental outline of 3-month dietary toxicology study in rats

Groups	Dose level (mg/kg/day)	No of animals							
		Total rats and sex		Toxicokinetic studies		Clinical pathology & necropsy		Microscopic	
				One month		3 months		Pathology	
		male	female	male	Female	male	female	male	female
1.Controls	0	10	10	-	-	10	10	10	10
2.	1	10	10	10	10	10	10	A.R.	A.R
3.	10	10	10	10	10	10	10	A.R.	A.R.
4.	40	10	10	10	10	10	10	10	10
5.	80	10	10	10	10	10	10	10	10
6.	120	10	10	10	10	10	10	A.R.	A.R.

A.R. = as required. Tissues from animals in group 2, 3 and 6 were examined if there were gross lesions present and/or if deemed necessary based on microscopic findings in groups 4 and 5.

Species/strain: Sprague-Dawley-derived CD rats; - Sprague-Dawley (SD)BR

Number/sex/group or time point (main study): Table 26

Route, formulation, volume, and infusion rate: oral, via dietary admixture

Satellite groups used for toxicokinetics: Table 26

Age: 40 days

Weight: 169.4-194.2g males; 127.1-151.1g females

Sampling times: animals were bled at the end of the fourth week at approximately 4, 8, 12, 16, 20 and 24 hr post dose for toxicokinetic study.

Mortality: twice daily

Clinical signs: twice daily

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: none

EKG: none

Hematology: day 92

Clinical chemistry: day 92

Urinalysis: none

Gross pathology: day 92

Organ weights: Table 27

Histopathology: Adequate Battery: yes, Table 27, Peer review: yes

Table 27
Tissues preserved and examined histopathologically

Organ name	No. examined	weighed	preserved	examined
Adrenal gland	2	x	x	x
Aorta (thoracic)	1		x	x
Bone (sternum)	2		x	x
Bone marrow (sternum)	2		x	x
Brain (medulla/pons, cerebrum and cerebellum)	3	x		x
Esophagus	1		x	x
Eyes with optic nerve	2		x	x
Heart	1	x	x	x
Kidneys	2	x	x	x
Lacrimal gland	2		x	x
Large intestine (cecum, colon, rectum)	3		x	x
Liver	2	x	x	x
Lungs (with mainstem bronchi)	2		x	x
Lymph node (mesenteric)	1		x	x
Mammary gland	1		x	x
Nerve (sciatic)	1		x	x
Ovary	2	x	x	x
Pancreas	1		x	x
Pituitary gland	1	x	x	x
Prostate gland with seminal vesicles	2	x	x	x
Salivary gland (submandibular)	2		x	x
Skeletal muscle (biceps femoris)	1		x	x
Skin	1		x	x
Small intestine (duodenum, ileum, jejunum)	3		x	x
Spinal cord (cervical, thoracic, lumbar)	3		x	x
Spleen	1	x	x	x
Stomach	1		x	x
Testes with epididymides	4	x	x	x
Thymic region	1	x	x	x
Thyroid/parathyroid glands	2	x	x	x
Trachea	1			
Urinary bladder	1			
Uterus (body/horns) with cervix	3	x		
Vagina	1			
Tissues with macroscopic findings, tissue masses				

Results

Mortality: all animals in the 120 mg/kg/day, all males and 6 females (80 mg/kg/day) and one female (40 mg/kg/day) died or were sacrificed moribund condition.

Clinical signs: some animals died exhibited antemortem abnormalities (decreased activity, pale appearance, labored breathing, irregular gait, hunched appearance and/or decreased fecal volume). Other observations were generally unremarkable.

Body weights: animals (120 mg/kg/day) generally exhibiting weight losses prior to death. Statistically significant decreases, relative to controls, were evident in mean body weights and cumulative weight changes for males receiving 10, 40 and 80 mg/kg/day. Time to onset and degree of difference from controls was dose related. Body weights for females receiving 10 mg/kg/day and males and female treated with 1 mg/kg/day were comparable to concurrent control values. At study termination, mean weights for males in the 10 and 40 mg/kg/day groups were 8% and 33% lower compared to the controls and mean weight for females in the 40 and 80 mg/kg/day groups were 13% and 33% lower than the controls.

Food consumption: mean food consumption values for males (80 and 120 mg/kg/day) and females (120 mg/kg/day) were statistically (p=0.01) lower than the controls throughout the period. Values for other treated groups were generally comparable to the controls.

Entecavir intake: approximate average entecavir intakes (based on food consumption data) over up to 13 weeks of treatment are as follows (Table 28).

Table 28
Approximate average entecavir intake

Group	Target dose (mg/kg/day)	Average intake (mg/kg/day)	
		Male	Female
2	1	1	0.99
3	10	10.0	9.94
4	40	19.71	39.71
5	80	72.13	76.75
6	120	101.35	103.49

Hematology: minimal elevations in total leukocyte counts occurred at 40 mg/kg/day (females) and 80 mg/kg/day. Fibrinogen values for females in the 40 and 80 mg/kg/day groups and platelet counts for males in the 40 mg/kg/day group were minimally to moderately higher than the controls.

Clinical chemistry: minimal to mild, dose related increased in serum AST and ALT occurred in all surviving groups of treated males (1, 10 and 40 mg/kg/day) and females (40 and 80 mg/kg/day). Mild elevations in serum cholesterol occurred in males in the 40 mg/kg/day and in females in the 10, 40 and 80 mg/kg/day groups. Mild to moderate decreases in serum ALP occurred at 40 and 80 mg/kg/day, as well as a minimal decrease in serum albumin in females. Other changes in surviving females (80 mg/kg/day) included decreases in serum glucose, total protein and albumin and alteration in some serum electrolytes.

Gross pathology: at 80 mg/kg/day, drug related gross lesions were seen in the duodenum which was noted to have thickened wall, red discoloration, adhesions, nodules or masses. This was correlated with necrosis and/or hemorrhage seen microscopically in the duodenum. The testes were noted to be small in 2/10 males; this was related to degeneration of the seminiferous tubular epithelium that was seen in 5 animals. At 120 mg/kg/day, several animals noted to have lesion in the duodenum at necropsy: abscesses,

adhesions, red discoloration, thickened wall, nodules or masses, which were seen in the 80 mg/kg/day groups of animals. Additionally, one male had small testes and four males had small seminal vesicles. In animals which survived to study termination, changes consisted of red discoloration of duodenum in one male (40 mg/kg/day) and 2 females (80 mg/kg/day).

Organ weights: several statistically significant differences from control values occurred in organ weight values for males in the 10 and 40 mg/kg/day groups and females in the 40 and 80 mg/kg/day groups. These differences were generally consistent with the statistically significant decreased body weight in these groups. These organs included spleen, testes and uterus. No drug related effects on organs were noted in males (1 mg/kg/day) or females (1 or 10 mg/kg/day).

Histopathology: Adequate Battery: yes, Peer review: yes

1 mg/kg/day: no drug related microscopic changes were observed.

10 mg/kg/day: drug related microscopic changes were limited to skeletal muscle myopathy in one male.

40 mg/kg/day: changes in terminal sacrificed animals were observed in the small intestine, skeletal muscle, lymph nodes and bone marrow. In addition, one unscheduled necropsied animals had microscopic changes in the thymus. One male had mild myeloid and erythroid depletion. One male had mild transmural hemorrhage and minimal mucosal necrosis in the distal duodenum. Eight males and 6 females (terminal sacrificed animals) had mostly minimal numbers of multinucleated giant cells in the lymph nodes. In the skeletal muscle, minimal myopathy was seen in three males and one female.

80 mg/kg/day: in both unscheduled necropsied and terminal sacrifice animals, changes were seen in the small intestine, lymph nodes, bone marrow and thymus. Additional drug related changes were seen in the heart, lungs, lymph nodes, spleen, kidneys, skeletal muscle and testes in unscheduled necropsied animals only.

In the small intestine, lesions occurred exclusively in the duodenum and consisted of mild to marked congestion, hemorrhage and/or necrosis. In the bone marrow, lesions considered of mostly moderate myeloid and erythroid depletion and/or congestion.

In the heart and lungs, microscopic lesions were present in unscheduled necropsied animals. Five male and two females had one or more fibrin thrombi present in pulmonary arterioles. Two of these males had moderate multifocal necrosis of the arteriolar media that was associated with the thrombi. Variable sized bacterial colonies that were present in the thrombi and scattered throughout the myocardium in several of these affected animals most likely represented postmortem bacterial overgrowth.

In the lymph nodes, lesions observed only in unscheduled necropsied animals included mild to moderate lymphoid depletion in 8 males and 5 females. In addition in both

unscheduled necropsied and terminal sacrificed animals, lesions included minimal to mild numbers of multinucleated giant cells of monocyte/macrophage in 3 males and 5 females.

In the spleen only in the unscheduled necropsied animals, lesions included mild to moderate lymphoid depletion in all males and 6 females. Lymphoid depletion occurred primarily in the periphery of the follicles in the white pulp.

In the thymus, in both unscheduled and scheduled sacrifices, lesions included mostly marked lymphoid atrophy in all males and seven females.

In the skeletal muscle, lesions were minimal to moderate myopathy characterized by degeneration and/or regeneration of individual muscle fibers in seven unscheduled necropsied males.

In the kidneys (5 unscheduled necropsied males), the lesions sere characterized by multifocal, mild to moderate tubular epithelial cell hyperplasia of proximal tubules in the outer medulla, in the area where proximal tubules meet the loop of Henle.

In the testes, lesions included multifocal, minimal to moderate degeneration of the seminiferous tubular epithelium in five unscheduled necropsied males.

120 mg/kg/day: no histopathology was carried out.

Toxicokinetics: data are shown in Table 29.

Table 29
Mean toxicokinetic parameters

Parameter	1 mg/kg/day		10 mg/kg/day		40 mg/kg/day		80 mg/kg/day		120 mg/kg/day	
	male	female	male	Female	male	female	male	female	male	female
Cmax (µg/ml)	0.017	0.011	0.073	0.095	0.417	0.32	1.594	0.797	3.21	2.37
Tmax (hr)	12	8	12	12	12	4	4	4	24	24
AUC (µg*hr/ml)	0.159	-	2.34	1.23	6.08	4.63	18.028	10.086	45.944	39.975

Study title: 6. Entecavir: Three-month oral toxicity in dogs

Key study findings: Groups of male and female beagle dogs (3 animals/sex/group) received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) for 3 consecutive months. The high dose was lowered from 30 to 15 mg/kg starting on day 29. Inflammation of the central nervous system (CNS) was seen clearly in dose related manner. At the low dose, minimal inflammation in the brain occurred in one of six dogs. At the mid dose, minimal to mild brain inflammation occurred in two of six dogs and minimal spinal cord inflammation occurred in one of these dogs. At the high dose, three dogs that survived had mild to moderate brain inflammation and two of these dogs also had minimal to mild spinal cord inflammation. In these dogs, there were no overt CNS signs at any dose levels. No CNS

changes were seen in a previous 2-week study in dogs. In 3-month rodent studies, histopathology results did not reveal any evidence of adverse effects on the CNS. The sponsor believed that the CNS inflammation may be a species specific finding in dogs. Rapidly dividing cells such as bone marrow and testes are the target organs for entecavir. In this study for CNS inflammation, a NOAEL could not be identified; however for other organs a dose level of 0.3 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be less than 0.05 mg/kg/day (2.9 mg/day for a 60 kg person). In this study at the NOAEL, the exposure was (AUC_{24ss}) was 1.2 and 1.3 μg*hr/ml in male and female dogs, respectively.

Study no.: 910067089/97202

Volume # and page #: 1 and 1-341

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: December 8, 1997

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # RO22A —

Methods

Doses: Groups of male and female beagle dogs (3 animals/sex/group) received repeated oral doses of entecavir capsules at dose levels of 0 (vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) for 3 consecutive months. The high dose was lowered from 30 to 15 mg/kg starting on day 29.

Species/strain: male and female beagle dogs

Number/sex/group or time point (main study): 3 animals/sex/group

Route, formulation, volume, and infusion rate: oral capsules

Satellite groups used for toxicokinetics: no

Age: 9 months

Weight: 8.8-12.1 kg

Sampling times: Blood samples for toxicokinetic investigations were collected at 0.5, 1, 2, 4, 6, 8 and 24 hr post dosing on days 1, 22 and 85. Plasma concentrations of entecavir were determined by a validated LC/MS method.

Mortality: once daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: daily

Ophthalmoscopy: during the 13th week daily

EKG: 13th week daily

Hematology: week 6 and 13th

Clinical chemistry: week 6 and 13th

Gross pathology: unscheduled and scheduled sacrificed animals

Organ weights: Table 30

Histopathology: Adequate Battery: yes, Table 30; Peer review: yes

Table 30
Tissues preserved and examined histopathologically

Organ name	weighed	examined
Adrenal gland	x	X
Aorta		X
Bone		X
Bone marrow (sternum)		X
Brain	x	X
Esophagus		X
Eyes with optic nerve		X
Gallbladder		
Heart	x	X
Kidneys	x	X
Large intestine (cecum, colon, rectum)		X
Liver	x	X
Lungs (with mainstem bronchi)		X
Lymph node (mesenteric)		X
Mammary gland		X
Ocular accessory glands		X
Ovary and oviducts	x	X
Pancreas		X
Pituitary gland	x	X
Prostate gland with seminal vesicles	x	X
Salivary gland (submandibular)		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
Spinal cord (cervical, thoracic, lumbar)		X
Spleen	x	X
Stomach		X
Testes with epididymides	x	X

Thymus		X
Thyroid/parathyroid glands	x	X
Trachea		X
Urinary bladder		X
Uterus (body/horns) with cervix		X
Vagina		X
Tissues with macroscopic findings, tissue masses		X

Results

Mortality: no deaths occurred at the low or mid dose. High dose: one male and two female dogs were sacrificed in moribund condition in week 5; this prompted the lowering of the dose to 15 mg/kg/day on day 29.

Clinical signs: there were no drug related signs of toxicity in dogs at the low dose. At the mid dose, reddened conjunctivae were seen in one female during the final 2 week of the study and decreased sizes of both testes were noted in one male in week 13. At the high dose, drug related clinical signs were seen in dogs sacrificed in week 5 consisted of decreased activity, thin appearance, salivation, hunched posture, increased rectal temperature and/or reduced turgor of skin. Drug related changes in the surviving dogs (high) occurred during weeks 2 to 6 and consisted of reddened conjunctivae, ocular discharge and ulceration of the glans penis (one dog); some of these changes were also observed in dogs that were sacrificed. No drug related ophthalmoscopic or EKG changes were seen at any dose level.

Body weights and Food consumption: no drug related changes in body weight or food consumption were seen at the low or mid dose. For weeks 5 and 6 (high), body weight loss and a moderate decrease in food consumption were observed. These changes were largely attributable to those dogs that were sacrificed. Body weight and food consumption values in the surviving dogs (high) were comparable to the controls from week 6 until end of the dosing period.

Hematology: no drug related changes were seen at the low or mid dose. Changes seen in one or more moribund animals (high) were consistent with an effect on the bone marrow and/or poor condition of dogs and included mild anemia; mildly increased erythrocytic parameters; marked decreases in reticulocyte, leukocyte and platelet counts; and moderately decreased APTT and fibrinogen. Changes seen in the surviving dogs (high) consisted of mild to moderate decreases in hemoglobin, hematocrit and erythrocyte, total leukocyte, lymphocyte, neutrophil, eosinophil and platelet counts in week 6. At week 13, only mild to moderate decreases in total leukocyte, lymphocyte and neutrophil counts were evident. In these dogs, there was an increased myeloid/erythroid ratio, moderately reduced numbers of megakaryocytes and minimally increased number of giant granulocytic precursor cells.

Clinical chemistry: no drug related changes were seen at the low or mid dose. At the high dose, marked increases in serum ALP was seen in one of the moribund female dog. Other change of lesser severity observed in one or more dogs sacrificed in moribund condition included increases in serum triglycerides, cholesterol, total protein, globulins, AST and urea nitrogen, and alteration in serum electrolytes. In the surviving dogs (high),

changes included mild increases in serum globulins and ALP at week 6 and a mild increase in serum GGT at week 13. Other changes included decreased serum creatinine, serum triglycerides, and increased serum potassium and bilirubin.

Urinalysis: no drug related changes were observed.

Gross pathology: **in unscheduled necropsied animals (high),** pale discoloration of the kidney and liver, pale discoloration of the oral mucosa; ulceration of the prepuce and decreased prostate size of one male; and multifocal ulceration of the jejunal and ileal mucosa in one female. In addition, red discoloration and/or adherent material abound the eyelids, general dehydration and adherent material and dryness of the nares were seen in these dogs.

End of dose sacrificed animals: gross changes were confined to the male genital organs and consisted of decreased size of the testes (mid or high) and decreased size of the prostate in one male (high).

Organ weights: drug related decreases were noted for the testes and prostate at all dose levels and these changes correlated with decreased size of the testes (mid and high) and decreased size of the prostate (high). Decreased weights of the ovaries were observed in female dogs (low and high).

Histopathology: Adequate Battery: yes, Peer review: yes

Unscheduled necropsied animals (one male and two females at the high dose), drug related changes were seen the bone marrow, lymph nodes, thymus, large and small intestines, testes, prostate, kidney, liver and pancreas.

In the bone marrow, mild to marked depletion of the erythroid and myeloid cells was seen. Marked atrophy of the thymus and minimal to moderate lymphoid depletion in the mesenteric and mediastinal lymph nodes associated with histocytosis, pigment deposition and/or hemorrhage were observed. In the small intestines, there was dilatation of the crypts of Lieberkuhn and atrophy and necrosis of the glandular epithelium; with most severe changes in the proximal segments (duodenum and jejunum).

In the large intestines, there was minimal to mild dilatation of the intestinal glands and minimal to mild necrosis and/or minimal atrophy of the epithelial cells.

In the testes of males, marked diffuse degeneration of the seminiferous tubular epithelium, and moderate atrophy of the prostate gland were seen.

In the kidneys, mild multifocal or diffuse degeneration of the renal tubules was present.

In the liver, mild multifocal hypertrophy/hyperplasia of the Kupffer cells and mild sinusoidal dilatation were noted.

In the pancreas, mild to moderate depletion of secretory granules (zymogen) were present.

End of dose sacrificed animals: drug related microscopic inflammatory lesions were noted in the central nervous system at all dose levels.

Low dose (one male): drug related change consisted of minimal focal subacute inflammation of the choroid plexus in the brain. Mid dose (one male and one female): drug-related findings were minimal to mild perivascular subacute inflammation in the choroid plexus and neuropil, choroids plexus and/or meninges of the brain; in the female minimal subacute inflammation was also seen in the spinal cord. High dose (2 males and one female) mild to moderate perivascular subacute inflammation of the neuropil and choroids plexus was seen. Minimal to mild subacute inflammation of the spinal cord was also present in two dogs.

Other drug related lesions, similar to those in unscheduled necropsied animals, were seen in the bone marrow, lymph nodes, pancreas, prostate and testes of these dogs.

In the bone marrow (high), minimal depletion of the erythroid and myeloid cell lines was noted in one male; and the same animal had minimal lymphoid depletion in the cervical lymph nodes.

At the mid and high doses, subacute inflammation and depletion of the secretory zymogen granules was noted in both male and female dogs. Degeneration of the seminiferous tubules was noted in the testes of males and atrophy of the prostate was seen in one male and correlated with lower organ weights noted at necropsy.

Toxicokinetics: mean toxicokinetics parameters are shown in Table 31. Peak plasma levels of entecavir were achieved at median Tmax values of 0.5 to 2 hr. Toxicokinetic evaluations indicated that the animals received dose related but not necessarily dose proportional systemic exposure to drug. At low and mid dose, the mean exposure values on days 22 and 85 were higher than day 1 values.

Table 31
Summary of mean toxicokinetic parameters of entecavir in a 3-month toxicology study in dogs

Group; dosage (mg/kg/day)		AUC_{24} (µg*hr/ml)			Cmax (µg/ml)		
		drug days			drug days		
		1	22	85	1	22	85
low 0.3	male	0.7	1.7	1.2	0.2	0.3	0.2
	female	0.6	1.2	1.3	0.2	0.2	0.2
mid 3	male	10.5	13.5	10.8	2.3	2.9	2.1
	female	9.7	12.8	11.6	2.2	3	1.8
High 30/15	male	96	89	58	17.6	13.5	8.4
	female	123	98	59	22.7	20.2	9

Study title: 7. Entecavir: Six-month oral range finding in mice

Key study findings: Groups of male and female CD-1 mice (20 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (sterile water; vehicle control), 0.2 (low), 1 (mid) or 5 mg/kg/day (high) for a period of 6 months. At the low dose, the liver was the only target organ, and the changes observed in the liver were minimal in severity. At the mid dose, changes in the liver were also minimal/ in addition, minimal changes in skeletal muscle and the lungs were seen. At the high dose, minimal to mild changes in the liver and minimal to mild changes in skeletal muscle and lungs were evident. The liver, skeletal muscle and lungs were the target organs in this study. A dose level of 5 mg/kg/day (high) may be considered the MTD for an oral carcinogenicity study. In this study, a NOAEL could not be determined; however, it should be less than 0.2 mg/kg/day. In this study at the low dose, the exposure was (AUC_{24ss}) was 0.321 and 0.336 µg*hr/ml in male and female mice, respectively.

Study no.: (Study No. 97046)

Volume # and page #: 1 and 1-409

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: October 16, 1998

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R023A ——

Methods

Doses: oral gavage dose of entecavir at dose levels of 0 (1% sterile water; vehicle control), 0.2 (low), 1 (mid) or 5 mg/kg/day (high) for a period of 6 months.

Species/strain: male and female CD-1outbred albino mice

Number/sex/group or time point (main study): 25 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics or recovery: 15 animals/sex/group

Age: 6 weeks

Weight: 21-32 g for males and 19-26 for females

Sampling times: Plasma concentrations of entecavir were determined in at 0.5, 1, 2, 4, 8 and 24 hr after the 58th dose of the study.

Mortality: once a day

Clinical signs: once a day

Body weights: twice a week during the first month and once weekly thereafter

Food consumption: twice a week during the first month and once weekly thereafter

Ophthalmoscopy: during the third and sixth month daily

EKG: not done

Hematology: end of dosing period

Clinical chemistry: end of dosing period

Urinalysis: not done

Gross pathology: organ listed in Table 1 were weighed for animals survived to scheduled necropsies.

Organ weights: listed in Table 32

Histopathology: Adequate Battery: yes, Table 32, Peer review: yes

Table 32
Tissues preserved and examined histopathologically

Organ name	weighed	examined
Adrenal gland		X
Aorta		X
Bone		X
Bone marrow (sternum)		X
Brain	X	X
Esophagus		X
Eyes with optic nerve		X
Gallbladder		
Harderian glands		
Heart	X	X
Kidneys	X	X
Large intestine (cecum, colon, rectum)		X
Liver	X	X
Lungs (with mainstem bronchi)		X
Lymph node (mesenteric)		X
Mammary gland		X
Ocular accessory glands		X
Ovary	X	X
Pancreas		X
Pituitary gland	X	X
Peripheral nerve		
Prostate gland	X	X
Salivary gland		X
Skeletal muscle (thigh, diaphragm)		X
Skin (inguinal region)		X
Small intestine (duodenum, ileum, jejunum)		X
Spinal cord (mid thoracic area)		X
Spleen	X	X
Stomach (cardiac, fundic and pyloric regions)		X
Testes with epididymides	X	X
Thymus		X
Thyroid/parathyroid glands	X	X
Trachea		X
Urinary bladder		X
Uterus with cervix	X	X
Vagina		X
Tissues with macroscopic findings, tissue masses		X

Results

Mortality: number of deaths with an average of 4/group including the controls occurred during the first 16 days of the study that were attributed to dosing procedure difficulties. Additionally, two males (mid) were found dead on days 54 and 154. These two deaths were considered accidental by the sponsor since no other deaths occurred at the high dose.

Clinical signs: no drug related overt signs of toxicity were observed.

Body weights: no drug related changes were seen at the low or mid dose. At the high dose, additional drug related changes included: lower body weights in males (approximately 10% lower than controls).

Ophthalmoscopy: no changes were seen.

Hematology: no changes were seen at the low or mid dose. At the high dose, minimal to moderate decreases in total leukocyte and lymphocyte counts were observed.

Clinical chemistry: no drug related changes were seen at the low or mid dose. At the high dose, mild to moderate increases in serum ALT and AST in males (ALT<AST), mild to moderate increases in serum AST in two females and minimal decreases in serum cholesterol in females and total protein and albumin in males were observed.

Urinalysis: not done.

Gross pathology: no drug related gross lesions were observed.

Organ weights: drug related changes occurred only at the high dose and consisted of decreased testes and increased kidney (males) weights as compared to the controls. No histopathological correlates to these organs were observed.

Histopathology: drug related changes were seen in the liver in all treated groups and in the lung and skeletal muscle at the mid and high dose.

Liver: at all doses minimal to moderate centrilobular degeneration in the liver, characterized by single cell necrosis of hepatocytes, scattered Kupffer cells distended with greenish brown pigment and minimal mononuclear inflammatory cells in adjacent sinusoidal spaces. The greenish brown pigment in Kupffer cells was positive for fat and polysaccharides. Thus, the pigment was most likely oxidative breakdown products (lipofuscin and ceroid) from degenerating hepatocytes. Additional drug related lesions (high) were minimal to mild (one male) and included centrilobular hepatocellular hypertrophy.

Skeletal muscle: multifocal myopathy was seen in both the diaphragm and thigh (mid and high). The myopathy was minimal (mid, females only) and minimal to mild (high) and was characterized by degeneration and regeneration of individual muscle fiber.

Lungs: multifocal histiocytosis was observed at the mid and high doses. The histiocytosis was minimal at the mid dose and minimal to mild at the high dose and characterized by multifocal aggregates of large foamy macrophages (histiocytosis) within alveoli, frequently in subpleural locations.

Toxicokinetics: mean toxicokinetic parameters and cerebrospinal fluid concentrations of entecavir are shown in Table 33. There were no apparent exposure differences between males and females. Peak plasma concentrations were achieved at Tmax values of 0.5 hr. The daily systemic exposure of mice to entecavir generally increased with an increase in dose; although the increases in exposure were less that the dose increments.

Table 33
Mean toxicokinetic parameters of entecavir in mice

Group; dosage (mg/kg/day)		Plasma				CSF	
		AUC_{24} (µg*hr/ml)		Cmax (µg/ml)		Mean concentration (ng/ml)	
		male	female	Male	female	male	female
0.2, low	58	0.321	0.336	0.07	0.056	8	13
1, mid	58	0.313	1.20	0.21	0.146	27	33
15, high	58	1.18	1.27	0.76	1.10	75	39

Study title: 8. Entecavir: Six-month oral toxicity in rats

Key study findings: Groups of male and female rat (20 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (sterile water; vehicle control), 0.6 (low), 3 (mid) or 15 mg/kg/day (high) for a period of 6 months. The target organs were liver and skeletal muscle at all doses tested. Degenerative changes in the liver associated with enlarged hepatocellular mitochondria and skeletal muscle myopathy were evident at all doses tested in this study. In a previous 3-month dietary study in rats, there were no drug related changes in the liver at the overtly toxic dose of 80 mg/kg/day and the threshold dose for skeletal muscle myopathy was 10 mg/kg/day. Thus, the toxicity of entecavir increased when the dosing period was extended beyond 3 months. In this study, a NOAEL could not be determined; however, it should be less than 0.6 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be less than 0.097 mg/kg/day (5.8 mg/day for a 60 kg person). In this study at the low dose, the exposure was (AUC_{24ss}) was 0.408 and 0.257 µg*hr/ml in male and female rats, respectively.

Study no.: 97039

Volume # and page #: 1 and 1-848

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: September 9, **1998**

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R022A, —

Methods
Doses: dose levels of 0 (1% sterile water; vehicle control), 0.6 (low), 3 (mid) or 15 mg/kg/day (high) for a period of 6 months.

Species/strain: male and female rats; strain: Sprague Dawley outbred

Number/sex/group or time point (main study): 20 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 0.4 to 10 mg/kg

Satellite groups used for toxicokinetics: 20 rats/sex/group

Age: 5 weeks

Weight: 115-133 g for males and 98-117 g for females

Sampling times: Plasma concentrations of entecavir were determined at 0.5, 1, 2, 4, 8 and 24 hr after the first, 85th and 176th doses of the study.

Mortality: twice daily

Clinical signs: twice daily

Body weights: twice each week during first month and once each week thereafter

Food consumption: once weekly

Ophthalmoscopy: weeks 11 and 24

Hematology: week 11 and 25

Clinical chemistry: weeks 11 and 25

Urinalysis: weeks 10 and 23

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: listed in Table 34

Histopathology: Table 34, Adequate Battery: yes, Peer review: yes

Table 34
Tissues preserved and examined histopathologically

Organ name	weighed	examined
Adrenal gland	X	X
Aorta		X
Bone		X
Bone marrow (sternum)		X
Brain	x	X
Esophagus		X
Eyes		X
Harderian glands		x

Heart	x	X
Kidneys	x	X
Large intestine (cecum, colon)		X
Liver	x	X
Lungs (with mainstem bronchi)		X
Lymph node (mesenteric)		X
Mammary gland		X
Ocular accessory glands		X
Ovary	x	X
Pancreas		X
Pituitary gland	x	X
Peripheral nerve		x
Prostate gland	x	X
Salivary gland		X
Skeletal muscle (thigh, diaphragm)		X
Skin (inguinal region)		X
Small intestine (duodenum, ileum, jejunum)		X
Spinal cord (mid thoracic area)		X
Spleen	x	X
Stomach (cardiac, fundic and pyloric regions)		X
Testes with epididymides	x	X
Thymus	x	X
Thyroid/parathyroid glands	x	X
Tongue		x
Trachea		X
Urinary bladder		X
Uterus with cervix	x	X
Vagina		X
Tissues with macroscopic findings, tissue masses		X

Results

Mortality: three control male rats died on days 20, 73 and 80 each. There were no deaths at the low or mid dose level. At the high dose, one male (day 44) and two females died on days 154 and 128. Pathologic examination of these animals showed that all deaths were consistent with dosing accidents, except the male (high) for which a cause of death could not be determined.

Clinical signs: There were no drug related clinical signs of toxicity, no changes in condition or behavior or ophthalmoscopic finding were noted at any dose.

Body weight and food consumption: no changes were seen (low or mid) animals. At the high dose, body weight gain was decreased (23%) in males; in addition mild weight loss was noted in some female animals. No drug related changes were seen in food consumption at the low or mid dose. At the high dose, food consumption was sporadically lower in males throughout the study.

Hematology: no drug related changes were observed at the low or mid dose. At the high dose, changes consisted of a mild increase in total leukocyte counts in males and minimal to mild increase in prothrombin time.

Clinical chemistry: drug related minimal to mild decreases in serum total protein, albumin and globulins in males were noted at all dose levels. At the mid and low doses, minimal to mild increases in serum urea nitrogen and cholesterol and minimal to

moderate increases in serum AST were seen in males. At the high dose in females, changes included mild increases in serum urea nitrogen, cholesterol, and sodium; a minimal increase in serum AST, and minimal to mild increases in serum ALT in males (ALT<AST); minimal increases in serum calcium and minimal to mild increases in serum chloride; and a minimal decrease in serum potassium in males.

Urinalysis: no drug related changes.

Gross pathology: drug related changes were limited to decreased testes size in seven males at the high dose.

Organ weights: drug related organ weight changes were limited to decreases in testes weights at the high dose level, which correlated grossly with decreased size of the testes.

Histopathology: drug-related microscopic changes were seen in the liver and skeletal muscle in all treated groups (low, mid or high) with dose-related increases in incidence.

Liver: lesions consisted of centrilobular liver degeneration in male and female rats (low, mid or high). The liver degeneration was oriented predominantly around the central veins, often with bridging between adjacent lobules and was characterized morphologically by single cell necrosis of hepatocytes. Kupffer cells distended with greenish pigment and variable numbers of mononuclear inflammatory cells were present in adjacent sinusoidal spaces. The greenish pigment present in Kupffer cells, which was positive for glycoprotein and was most likely breakdown products from degenerating hepatocytes. There was no drug related differences in hepatocytes fat or glycogen contents between the controls and treated animals. However, the fat present in the liver of females (mid or high) was distributed mostly in a centrilobular pattern, compared to more diffuse distribution observed in controls.

Skeletal muscle: myopathy occurred in all treated groups (low, mid or high) with dose related increases in incidence. This lesion was mostly minimal in all dose groups and was characterized by variable numbers of individual muscle fibers undergoing degeneration and/or regeneration. The myopathy was observed most commonly in hind-limb skeletal muscle in all dose groups; however, the myopathy was also observed in the diaphragm (males) and the tunica muscularis of the esophagus (high).

Toxicokinetics: evaluations on days 1, 85 and 176 revealed that systemic exposures to drug increased with dose and there were no distinct exposure differences after multiple dosing. Males had higher AUC values than females (Table 35). Peak plasma levels were achieved between 0.5 and 2 hr. There was no distinct trend with regard to exposure differences after multiple dosing. After 6 month of dosing, mean cerebrospinal fluid concentrations of entecavir ranged from approximately 0.03 to 0.258 μg/ml (Table 36). Overall, the results indicated that there were dose related systemic exposures of rat to entecavir in the 6-month oral toxicity study.

Table 35
Summary of mean toxicokinetic parameters of entecavir in rats

Group; dosage (mg/kg/day)		AUC_{24} (µg*hr/ml)			Cmax (µg/ml)		
		drug days			drug days		
		1	85	176	1	85	176
low 0.6	male	0.25	0.47	0.4	0.06	0.072	0.09
	female	0.17	0.42	0.25	0.06	0.04	0.046
mid 3	male	1.4	2.3	2.1	0.4	0.4	0.4
	female	1.1	1.2	1.0	0.36	0.25	0.21
High 15	male	8.9	7.9	6.3	2.1	1.2	1.1
	female	5.6	2.4	4.5	1.6	0.57	0.98

Table 36
Mean plasma and cerebrospinal fluid concentrations of entecavir in rats

Group; dosage (mg/kg/day)		Plasma (µg/ml)	CSF (µg/ml)	CSF: Plasma ratio
		Drug day		
		176	176	
low 0.6	Male	0.069	0.03	0.13
	Female	0.046	0.18	0.39
mid 3	Male	0.399	0.092	0.23
	Female	0.191	0.108	0.57
High 15	Male	0.936	0.184	0.2
	female	0.833	0.258	0.31

Study title: 9. Entecavir: Six-month oral toxicity in rats

Key study findings: groups of male and female rats (20 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (sterile water; vehicle control), 0.02 (low), 0.08 (mid) or 0.3 mg/kg/day (high) for a period of 6 months. The target organ was liver; no drug related lesions were seen in the skeletal muscle. Incidence of minimal centrilobular degeneration in the liver was 4 out of 40 rats (low). The low incidence at 0.02 mg/kg/day suggested that this dose is at or near the threshold dose for effects on the liver. A dose level of 0.3 mg/kg/day may be considered a NOAEL for skeletal muscle. In this study, a NOAEL for general toxicology could not be determined.

In this study at the low dose, the exposure was (AUC_{24ss}) was 8.06 and 4.1 ng*hr/ml in male and female rats, respectively.

Study no.: 98044

Volume # and page #: 1 and 1-524

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: December 8, 1999

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R022A. —

Methods

Doses: animals received once daily oral gavage dose of entecavir at dose levels of 0 (1% sterile water; vehicle control), 0.02 (low), 0.08 (mid) or 0.3 mg/kg/day (high) for a period of 6 months.

Species/strain: male and female rats; strain: — Sprague Dawley outbred

Number/sex/group or time point (main study): 20 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, 1 ml/kg

Satellite groups used for toxicokinetics: 10 animals/sex/group

Age: 5 weeks

Weight: 95-135 g for males and 92-120 g for females

Sampling times: Plasma concentrations of entecavir were determined at 0.5, 1, 2, 4, 8 and 24 hr after the first, 91st and 171st doses of the study.

Mortality: twice daily

Clinical signs: twice daily

Body weights: twice each week during first month and once each week thereafter

Food consumption: once weekly

Ophthalmoscopy: week 26

Hematology: week 13 and 26

Clinical chemistry: weeks 13 and 26

Urinalysis: not done

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: not done

Histopathology: Table 37. Adequate Battery: yes, Peer review: yes

Table 37
Tissues histopathologically examined and preserved

Organ name	examined
Adrenal gland	X
Aorta	X
Bone	X
Bone marrow (sternum)	X
Brain	X
Esophagus	X
Eyes	X
Harderian glands	x
Heart	X
Kidneys	X
Large intestine (cecum, colon)	X
Liver	X
Lungs (with mainstem bronchi)	X
Lymph node (mesenteric)	X
Mammary gland	X
Ocular accessory glands	X
Ovary	X
Pancreas	X
Pituitary gland	X
Peripheral nerve	x
Prostate gland	X
Salivary gland	X
Skeletal muscle (thigh, diaphragm)	X
Skin (inguinal region)	X
Small intestine (duodenum, ileum, jejunum)	X
Spinal cord (mid thoracic area)	X
Spleen	X
Stomach (cardiac, fundic and pyloric regions)	X
Testes with epididymides	X
Thymus	X
Thyroid/parathyroid glands	X
Tongue	x
Trachea	X
Urinary bladder	X
Uterus with cervix	X
Vagina	X
Tissues with macroscopic findings, tissue masses	X

Results

Mortality: there were no drug related deaths.

Clinical signs: There were no drug related clinical signs of toxicity, changes in condition or behavior or ophthalmoscopic finding noted at any dose.

Body weight and food consumption: no changes were seen (low or mid) dose animals. At the high dose, a transient minimal decrease in body weight was evident during the middle of the study. There were no changes in food consumption.

Hematology: no drug related changes were observed.

Clinical chemistry: no drug related changes were seen at the low dose. At the high dose, increases in serum ALT and AST were observed in one male at week 13 and slight increases in serum sodium and/or chloride were evident at the mid or high dose rats during the study.

Urinalysis: no effects

Gross pathology: no drug related changes were seen.

Organ weights: not done.

Histopathology: Table 37. Adequate Battery: yes, Peer review: yes

Drug related microscopic changes in the liver were seen in all treated groups. No drug related microscopic changes were seen in the skeletal muscle any dose level.

Liver: drug related lesions consisted of dose related increasing incidences of centrilobular liver degeneration minimal in severity at all doses. The liver degeneration was oriented around the central veins and was characterized by single cell necrosis of hepatocytes, Kupffer cells containing small amounts of greenish brown pigment and small number of mononuclear inflammatory cells in adjacent sinusoidal spaces. These changes were similar to those observed in a previous 6-month study in rats. The greenish brown pigment present in Kupffer cells was consistent with breakdown products (lipofuscin) from degenerating hepatocytes.

Toxicokinetics: evaluations on days 1, 91 and 171 revealed that systemic exposures to drug increased with dose and there were no distinct exposure differences after multiple dosing. Males had higher AUC values than females (Table 38). Toxicokinetic evaluations revealed that there were dose related systemic exposures to entecavir in this study. At the low dose, steady state exposures to entecavir were lower compared to day 1. Tmax ranged from 0.5 to 4 hr.

Table 38
Summary of mean toxicokinetic parameters of entecavir in rats

Group; dosage (mg/kg/day)		AUC_{24} (μg*hr/ml)				Cmax (ng/ml)	
		drug days				drug days	
		1	91	171	1	91	171
low 0.02	Male	0.037	0.026	0.008	10.2	2.1	1.7
	Female	0.016	0.006	0.004	17.2	3.6	1.4
mid 0.08	male	0.043	0.049	0.05	19.8	5.9	4.3
	female	0.017	0.018	0.030	7.5	5.5	4.1
High 0.3	male	0.131	0.149	0.168	44.3	24.1	21.5
	female	0.115	0.118	0.117	20.1	41.7	17.6

Study title: Entecavir: 10. One-month oral range finding toxicity study in monkeys

Key study findings: Groups of male and female cynomolgus monkeys (2 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (1% sodium carboxymethylcellulose; vehicle control), 5 (low) or 25 mg/kg/day (high) for a period of one month. No drug related clinical signs or effect on body weights or food consumption were noted. No drug related changes in hematological or serum chemistry parameters were observed. No drug related gross lesions were seen. Drug related microscopic findings were limited to liver in both groups of monkeys and consisted of minimal to mild hepatocellular cytoplasmic vacuolation.

In this study, a NOAEL could not be identified. Exposure (AUC_{ss}) was 0.655 and 2.24 μg*hr/ml at the low and high doses, respectively.

Study no.: 95721

Volume #, and page #: 1, 1-46

Conducting laboratory and location: Bristol-Myers Squibb

Date of study completion: June 14, 1996

GLP compliance: No

QA report: No

Drug, lot #, and % purity: 004

Methods

Doses: daily oral gavage dose of entecavir at dose levels of 0 (1% sodium carboxymethylcellulose; vehicle control), 5 (low) or 25 mg/kg/day (high) for a period of one month.

Species/strain: male and female cynomolgus monkeys

Number/sex/group or time point (main study): 2 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, 5 ml/kg

Satellite groups used for toxicokinetics or recovery: treated monkeys

Age: 3-4 years

Weight: 2.7-3.3 kg

Sampling times: plasma concentrations of entecavir were determined at 0.5, 1, 2, 4 and 8 hr on days 1 and 29 of the study by a validated LC/MS/MS method.

Mortality: 4-6 times daily

Clinical signs: 4-6 times daily

Body weights: 2-3 times weekly

Food consumption: 2-3 times weekly

Hematology: days 16 and 28

Clinical chemistry: days 16 and 28

Gross pathology: day 30

Histopathology: Adequate Battery: Table 39, yes, Peer review: no

Table 39
Tissues preserved and examined pathologically

Organ name	Gross pathology	Histopathology
Adrenal gland	X	X
Colon	X	X
Duodenum	X	X
Heart	X	X
Ileum	X	X
Iliac lymph node	X	X
Kidney	X	X

Liver	X	X
Lung	X	X
Mandibular lymph node	X	X
Mesenteric lymph node	X	X
Pancreas	X	X
Thyroid/parathyroid gland	X	X
Prostate	X	X
Uterus	X	X
Spleen	X	X
Sternum	X	X
Stomach	X	X
Testes or ovary	X	X
Thymus	X	X
Urinary bladder	X	X
Significant gross lesion	X	X

Results

Mortality: no deaths

Clinical signs: there were no drug related clinical signs of toxicity, changes in condition or behavior were noted at any dose.

Body weights: no effects on body weights or body weight gains were noted.

Food consumption: no changes were noted.

Hematology: no changes were noted.

Clinical chemistry: no changes were noted.

Gross pathology: no drug related lesions were found.

Histopathology: Adequate Battery: yes Peer review: no

Drug related microscopic changes were limited to the liver. Minimal to mild cytoplasmic vacuolation of centrilobular hepatocytes was observed in monkeys treated with either low or high dose. Hepatocellular vacuolation was characterized by a minimal increase in cell size, increased cytoplasmic pallor and fine, multiple, clear cytoplasmic vacuoles. This change was not typical of fatty change. A clear dose related increase in severity was not apparent.

Toxicokinetics: data are shown in Table 40. Exposures to entecavir increased in a dose related but generally less than dose proportional manner.

Table 40
Summary of mean toxicokinetic parameters in monkeys

Dose (mg/kg/day)	Cmax (ng/ml)		AUCss (ng*hr/ml)	
	Day 1	Day 29	Day 1	Day 29
5	149	186	416	655
25	585	578	2226	2246

Study title: Entecavir: 11. One-year oral toxicity in monkeys

Key study findings: Groups of male and female cynomolgus monkeys (6 animals/sex/group) received once daily oral gavage dose of entecavir at dose levels of 0 (avicel/methocel/water preparation; vehicle control), 0.4 (low), 4 (mid) or 40 mg/kg/day (high). After 3 month of dosing, two males and two females from each group were sacrificed for an interim evaluation; the remaining monkeys were dosed for 12 months. No drug related changes were seen at the low or mid dose. At the high dose males, minimally elevated mean serum urea nitrogen and potassium values were noted. Toxicokinetic evaluations indicated that there were dose related exposures to entecavir. No substantive differences in exposure to entecavir were evident between males and females. Mean CSF levels of entecavir were also dose related.

In this study, no substantive toxic effects were seen in monkeys given up to 40 mg/kg/day for one year. A dose of 40 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 13.33 mg/kg/day (800 mg/day for a 60 kg person). In this study at the NOAEL, exposure (AUC_{24ss}) was 4.64 and 3.15 μg*hr/ml in male and female monkeys, respectively.

Study no.: 97049/920000086

Volume #, and page #: 1, 1-771

Conducting laboratory and location: —

Date of study completion: June 30, 1999

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: **R023B and R022A.** —

Methods

Doses: daily oral gavage dose of entecavir at dose levels of 0 (avicel/methocel/water preparation; vehicle control), 0.4 (low), 4 (mid) or 40 mg/kg/day (high) for a period of 12 months. After 3 month of dosing, two males and two females from each group were

sacrificed for an interim evaluation; the remaining monkeys were dosed for 12 months.

Species/strain: male and female cynomolgus monkeys

Number/sex/group or time point (main study): 6 animals/sex/group

Route, formulation, volume, and infusion rate:

Satellite groups used for toxicokinetics or recovery: treated monkeys

Age: 3 years

Weight: 2.6-3.7 kg for males and 2.1 to 3.4 kg for females

Sampling times: plasma concentrations of entecavir were determined at 0.5, 1, 2, 4, 8 and 24 hr after the first, 85th, 180th and 365th doses of the study by a validated LC/MS/MS method.

Mortality: daily

Clinical signs: daily

Body weights: terminally

Food consumption: 5 times weekly

Ophthalmoscopy: weeks 4, 12, 24 and day 364

EKG: weeks 1, 4, 12, 26 and 52

Hematology: days 8, 27, 90, 176 and 363

Clinical chemistry: days 8, 27, 90, 176 and 363

Urinalysis: days 26, 27, 28, 29, 89, 90, 91, 175, 176, 177, 178, 362, 363, 364 and 365

Gross pathology: necropsy intervals: 3-month interim, test day 92; 12-monht terminal, test day 369-370

Organ weights: listed in Table 41

Histopathology: Adequate Battery: Table 41, yes, Peer review: yes,

Table 41
Tissues preserved and examined histopathologically

Organ name	weighed	examined
Adrenal gland	X	X
Aorta (abdominal)		X
Bone (sternum/femur)		X
Bone marrow (sternum/femur)		X
Brain (medulla/pons,cerebrum and cerebellum)	x	X
Esophagus		X
Eyes (with optic nerve)		X
Gall bladder		x
Heart	x	X
Kidneys	x	X
Lacrimal gland		x
Large intestine (cecum, colon and rectum)		X
Liver	x	X
Lungs (with mainstem bronchi)	x	X
Lymph node (mesenteric and mediastinal)		X
Mammary gland		X
Muscle (biceps femoris)		x
Nerve (sciatic)		X
Ovary	x	X
Pancreas		X
Pituitary gland	x	X
Peripheral nerve		x
Prostate gland	x	X
Salivary gland (submandibular)		X
Seminal vesicles	x	x
Skin (inguinal region)		X
Small intestine (duodenum, ileum, jejunum)		X
Spinal cord (cervical, thoracic and lumbar)		X
Spleen	x	X
Stomach		X
Testes with epididymides	x	X
Thymic region	x	X
Thyroid/parathyroid glands	x	X
Tongue		x
Trachea		X
Urinary bladder		X
Uterus with cervix		X
Vagina		X
Tissues with macroscopic findings, tissue masses		X

Results

Mortality: One female (mid) animal was euthanized in moribund condition on day 214. Postmortem examination determined meningoencephalitis to be the cause of the moribund condition. All other animals survived the treatment.

Clinical signs: there were no drug related clinical signs of toxicity, changes in condition or behavior, ophthalmoscopic or cardiology finding noted at any dose.

Body weights: no effects on body weights or body weight gains were noted. Body weight patterns in the controls and treated animals were similar.

Food consumption: no changes were noted.

Ophthalmoscopy: revealed no effects of entecavir administration.

EKG: no effects were noted.

Hematology: no changes were noted.

Clinical chemistry: no changes were noted for the low or mid dose. At the high dose, minimally elevated (statistically significant) mean values for serum urea nitrogen (BUN) and potassium, relative to the controls, were observed after 3 months in male monkeys. At six months, mean BUN values were comparable to the controls; at 12 months, they were slightly but statistically significantly elevated (high, 26%). Serum potassium values for males (high) were slightly higher than the controls at 3 months (12%), 6 months (18%) and 12 months (10%) evaluations.

Urinalysis: were unremarkable.

Gross pathology: no drug related lesions were found.

Organ weights: no effects were seen at the three month interim sacrifice. At 12 months, the mean brain weight of males (high) was slightly lower (14%) than the controls. No microscopic changes were seen in the brain.

Histopathology: Adequate Battery: yes Peer review: yes

At the 3-month interim sacrifice, microscopic findings occurred with comparable incidence or severity or they occurred sporadically in the control and treated groups. Examples of these spontaneously occurring findings were hepatocellular vacuolation in the liver, dilated lacteal in the small intestine, evidence of sexual immaturity and perivascular macrophage infiltration in the lungs. These incidental findings were not considered related to drug and have been seen in monkeys of similar age used in other studies by the sponsor.

Moribund sacrificed animal: a severe diffuse inflammatory process was seen involving all meningeal surfaces of the brain with extension into the brain parenchyma primarily along the pia-arachnoid into perivascular spaces.

Terminal sacrificed animals: microscopic findings occurred with comparable incidence or severity or they occurred in the controls and treated groups. Examples of these spontaneously occurring dings were hepatocellular vacuolation in the liver, dilated lacteals in the small intestine, evidence of sexual immaturity and perivascular macrophage infiltration in the lungs.

Toxicokinetics: data are shown in Table 42. Evaluations on days 1, 85, 180 and 365 revealed that systemic exposures to drug increased with dose and there were no distinct exposure differences after multiple dosing. No substantive differences in exposures were evident between males and females. Systemic exposure to entecavir was dose related but

les than dose proportional, with minimal accumulation. The mean Tmax for entecavir in plasma ranged from 0.5 to 5 hr. Mean cerebrospinal fluid concentrations of entecavir are summarized in Table 43.

Table 42
Summary of mean toxicokinetic parameters in monkeys

Group; dosage (mg/kg/day)		AUC_{24} (µg*hr/ml) drug days				Cmax (µg/ml) drug days			
		1	85	180	365	1	85	180	365
low 0.4	Male	0.047	0.052	0.071	0.08	0.01	.008	.009	.008
	Female	0.033	0.044	0.092	0.055	.005	.007	0.01	0.01
mid 4	Male	0.479	0.514	0.625	0.562	0.07	0.06	0.06	0.05
	Female	0.459	0.558	0.533	0.428	0.08	0.07	0.07	0.05
High40	Male	2.76	3.95	4.70	4.64	0.3	0.5	0.3	.35
	Female	2.04	2.75	2.47	3.15	0.3	0.3	0.3	.31

Table 43
Mean plasma and cerebrospinal fluid concentrations in monkeys

Group; dosage (mg/kg/day)		CSF conc. (ng/ml) Drug day 369	CSF: Plasma ratio
low 0.4	Male	50.51	8.48
	Female	0.59	0.1
mid 4	Male	4.08	0.09
	Female	1.42	0.03
High 40	Male	14.64	0.05
	Female	9.83	0.05

2.6.6.4 Genetic toxicology

Study title: 1. Entecavir: Ames reverse mutation study in Salmonella and Escherichia coli

Key findings: Entecavir was assessed for mutagenic potential in the Ames assay at concentrations up to 5000 µg/plate, both with or without S-9 metabolic activation. No

cytotoxicity was observed in any of the tester strains at any concentration, either with or without metabolic activation. The histamine+ and tryptophan+ revertant frequencies in each of the treated cultures were essentially at the concurrent negative controls. Under the conditions of this study, entecavir was not mutagenic in the Ames reverse mutation assay.

Study no.: 96669

Volume # and page #: 1 and 1-42

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: July 24, 1996

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Batch # N005A —

Methods

Strains/species/cell line: Table 44

Table 44
Entecavir concentrations tested for the Salmonella and Escherichia coli strains in both the presence and absence of metabolic activation

Test article	Dose level (μg/plate)	Salmonella strains				E. coli
		TA 98	TA 100	TA 1535	TA1537	WP2uvrA
DMSO, negative control	100 μl/plate	Yes	Yes	yes	yes	yes
entecavir	312.5	Yes	Yes	yes	yes	yes
	625	Yes	Yes	yes	yes	yes
	1250	Yes	Yes	yes	yes	yes
	2500	Yes	Yes	yes	yes	yes
	5000	Yes	Yes	yes	yes	yes
2-aminoanthracene, positive control	2.5	Yes	Yes	yes	yes	no
2-aminoanthracene, positive control	10	No	No	no	no	yes
2-nitrofluorene, positive control	2	Yes	No	no	no	no
Sodium azide, positive control	1	No	Yes	yes	no	no
9- aminoacridine, positive control	100	No	No		yes	no
Methylmethanesulfonate, positive control	25μl/plate	No	No	no	no	yes

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5

days prior to sacrifice.

Doses used in definitive study: Table 44

Basis of dose selection: a non-GLP exploratory study. In this study, no cytotoxicity was observed in the tested strains at any concentration up to the maximum of 4000 μg/plate.

Negative controls: Table 44

Positive controls: Table 44

Incubation and sampling times: after solidification of the agar overlay, all plates were incubated aerobically at 37 degrees in darkness for 46-48 hr.

Results

Entecavir was assessed for mutagenic potential in the Ames assay. Results of this evaluation are illustrated in Tables 45 and 46. No cytotoxicity was observed in any of the tester strains at any concentration, either with or without metabolic activation. The histamine+ and tryptophan+ revertant frequencies in each of the treated cultures were essentially at concurrent negative controls.

Table 45

Mean histidine+ and tryptophan+ revertant counts from the full assay on entecavir in the presence of metabolic activation

Test article	Dose level (μg/plate)	Salmonella strains				E. coli
		TA 98	TA 100	TA 1535	TA1537	WP2uvrA
DMSO	100	27	161	12	5	21
entecavir	312.5	31	142	12	4	17
	625	30	156	17	9	23
	1250	33	153	13	8	18
	2500	35	160	10	8	19
	5000	31	153	9	7	17
2-aminoanthracene	2.5	1552	1487	214	61	-
-do-	10	-	-	-	-	366

Table 46

Mean histidine+ and tryptophan + revertant counts from the full assay on entecavir in the absence of metabolic activation

Test article	Dose level (μg/plate)	Salmonella strains				E. coli
		TA 98	TA 100	TA 1535	TA1537	WP2uvrA
DMSO	100	24	129	15	4	17
entecavir	312.5	24	128	12	4	14
	625	32	153	9	8	17
	1250	30	152	9	9	18
	2500	27	140	9	5	19
	5000	30	137	15	7	13
2-nitrofluorene	2	696	-	-	-	-
Sodium azide	1	-	542	468	-	-
9- aminoacridine	100	-	-	-	515	-
Methylmethanesulfonate, positive control	2.5	-	-	-	-	573

Study validity: valid

Study outcome: entecavir was not mutagenic in the Ames assay.

Study title: 2. Entecavir: in vitro transformation of Syrian hamster embryo cells

Key findings: Primary cells from Syrian hamster embryos were exposed to entecavir for seven consecutive days at concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 µg/ml to evaluate morphologic transformations in the embryo. Also included in the study were the reference nucleosides acyclovir, ganciclovir and sorivudine, and a positive control, benzo(a)pyrene, a known rodent carcinogen. Results indicated no significant elevation in morphologically transformed colonies (MTCs) with entecavir. Of the three reference nucleosides, only sorivudine induced a statistically significant and toxicologically meaningful increase in MTCs. Under the conditions of this study, entecavir was negative in the Syrian hamster embryo cell transformation assay.

Study no.: 17974-0-485R

Volume #, and page #: 1 and 1-168

Conducting laboratory and location: [redacted]

Date of study completion: April 30, 1997

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: **Batch # [redacted] 14A,** [redacted]

Methods

Primary cells from Syrian hamster embryos were exposed to entecavir and reference agents continuously for 7 days (Table 47). Then, the cell colonies were evaluated for morphologic transformation. An increase in the frequency of morphologically transformed colonies (MTF) is considered predictive for carcinogenicity in rodent lifetime studies.

Table 47

List of test articles utilized and their concentrations tested in the Syrian hamster embryo in vitro morphologic transformation assay

Test articles	Concentrations (μg/ml)				
Entecavir	0.125	0.25	0.5	1.0	2.0
2-deoxyguansine	18	26	36	39	42
Ganciclovir	0.36	0.87	2.1	5	12
Acyclovir	0.35	0.50	0.75	1.1	1.65
Sorivudine	120	180	270	400	600
Medium, negative	-	-	-	-	-
DMSO, (0.2%)	-	-	-	-	-
Benzopyrene, positive control	1.25	2.5	-	-	-

Strains/species/cell line: Table 47

Doses used in definitive study: Table 47

Basis of dose selection: based on the results of a preliminary study, the doses listed in the Table 1 were selected.

Negative controls: Table 47

Positive controls: Table 47

Incubation and sampling times: the cultures were incubated in the presence of the test articles for period of approximately 7 days.

Results

Entecavir and four reference agents (ganciclovir, 2-deoxyguanosine, acyclovir and sorivudine: results are not shown in Table 48) and concurrent controls were evaluated for their potential to cause statistically significant increases in morphologic transformation frequencies. Only sorivudine showed a significant response at the dose levels tested. Entecavir, 2-deoxyguanosine, ganciclovir and acyclovir were negative with regard to their potential to cause morphologic transformation in Syrian hamster embryo cells.

Table 48

Overall summary of Syrian hamster embryo cell transformation of entecavir. Cells were continuously exposed for a period of 7 days

Test article	Concentration (μg/ml)	Total colonies	MT/MT freq.	Avg. P.E.	Relative P.E. (%)	P value, Fisher's
Entecavir	0.125	1059	4/0.378	31	94	0.5367
	0.25	1002	4/0.399	29	88	0.5704
	0.50	886	4/0.451	25	76	0.6174
	1.0	781	3/0.384	22	67	0.5709
	1.0 CA	1140	3/0.263	20	61	0.3553
	2.0	519	3/0.578	15	45	0.4886
	2.0 CA	1089	4/0.367	16	48	0.5195
Benzopyrene	1.25	1133	16/1.412	33	100	0.0136*
	2.5	1177	15/1.274	34	103	0.0259*
DMSO (0.2%)	-	1124	5/0.445	33	100	-
Medium	-	1097	3/0.2273	32	97	-

MT = combined total no. of morphological transformed colonies
MT Freq = MT/total colonies *100
Average P.E. = average P.E. of combined trial
CA = cell adjustment of dose equalized to controls
* = statistically significant treatment related difference between the morphologic transformation frequency (MTF) using 1-tailed Fisher's exact test.

Study validity : valid

Study outcome: Under the conditions of this study, entecavir was negative in the Syrian hamster embryo cell assay.

Study title: 3. Entecavir: Cytogenetic study in primary human lymphocytes

Key findings: Entecavir was tested for its potential to induce chromosome aberrations in cultured human lymphocytes with and without exogenous metabolic activation (rat liver homogenate from Aroclor-induced S-9 fraction) at dose levels of 2.5, 5, 10, 20, 50 or 200 μg/ml. Toxicity was assessed by measuring the reduction in the Relative Mitotic Index. The test article was tested with concurrent vehicle and positive controls. In this study, statistically significant elevations in the frequency of cells bearing metaphases chromosome aberration were seen at entecavir concentrations of 10 μg/ml or greater in the absence of S-9 enzymes and 50 μg/ml or greater in the presence of S-9 enzymes. Dose related and statistically significant decreases in mitotic index (cytotoxicity) were observed for cultures treated with entecavir in both the absence and presence of S-9 activation. Entecavir, at concentrations up to and including cytotoxic doses in both the absence and presence of S-9 metabolic activation, is clastogenic in the in vitro human lymphocyte chromosome aberration assay. Thus, it is concluded that entecavir is clastogenic to cultured human lymphocytes.

Study no.: 96668

Volume #, and page #: 1 and 1-36

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: September 19, 1996

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Batch # N005A. —

Methods

Experimental design of the chromosome aberration assay is shown in Table 49. At 5 hr exposure, the test articles were subjected to metabolic activation (S-9) fraction.

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5 days prior to sacrifice.

Table 49

Experimental design of cytogenetic study in primary human lymphocytes. At 5 hr exposure, test articles were treated with rat S-9 fraction (metabolic activation)

Test article	Dosing concentration (µg/ml)	5 hr incubation	24 hr incubation	Number of flasks
entecavir	2.5	Yes	yes	4
	5	-	yes	4
	10	Yes	yes	4
	20	-	yes	4
	50	Yes	-	4
	200	Yes	-	4
DMSO	10µl	Yes	yes	4
Mitomycin C	0.1		yes	4
Cyclophosphamide	4	Yes	no	4

Strains/species/cell line: primary human lymphocytes

Doses used in definitive study: Table 49

Basis of dose selection: in a dose range finding study, an assessment of chromosomal morphology and mitotic suppression was made at entecavir concentrations up to 2650 both without and with S-9 fraction activation. Based on the results of this study, doses were selected for the full chromosome aberration assay.

Negative controls: Table 49

Positive controls: Table 49

Incubation and sampling times: Table 49

Results:

Summaries of data are shown in Tables 50 and 51. In this study, statistically significant elevations in the frequency of cells bearing metaphases chromosome aberration were seen at entecavir concentrations of 10 μg/ml or greater in the absence of S-9 enzymes and 50 μg/ml or greater in the presence of S-9 enzymes. Dose related and statistically significant decreases in mitotic index (cytotoxicity) were observed for cultures treated with entecavir in both the absence and presence of S-9 activation.

Table 50

Summary of cytogenetic data of by dose group at 24 hr exposure without metabolic activation

Entecavir Conc. (μg/ml)	Mean mitotic index (%)	Cell with aberrations		Chromatid		Chromosome		Total aberrations
		% mean	ABS/cell	BKS	EXC	BKS	EXC	
2.5	9.1	3	0.03	4	0	2	0	6
5	6.9**	4	0.05	8	0	1	0	9
10	4.2**	16.5**	0.2	33	0	7	0	40
20	3.7**	35**	055	91	0	9	0	110
DMSO, vehicle	11.6	2	0.02	3	0	1	0	4
Mitomycin, positive control	6**	38.5**	0.64	87	19	21	1	128

* = significantly different from appropriate control at p=0.05
** = significantly different from appropriate control at p=0.01
BKS = total of all breaks
EXC = total of all exchanges

Table 51

Summary of cytogenetic data of by dose group at 5 hr exposure with metabolic activation (S-9)

Entecavir Conc. (μg/ml)	Mean mitotic index (%)	Cell with aberrations		Chromatid		Chromosome		Total ABS
		% mean	ABS/cell	BKS	EXC	BKS	EXC	
2.5	10.4	4.5	0.05	8	0	1	0	9
10	10	4.5	0.05	7	0	2	0	9
50	9.1	9.5*	0.1	14	0	5	0	19
200	4.9**	34**	0.66	96	4	21	1	132
DMSO, vehicle	11.4	4	0.04	7	0	1	0	8
Cyclophosphamide positive control	7.7*	36.5**	0.63	88	21	16	0	125

* = significantly different from appropriate control at p=0.05
** = significantly different from appropriate control at p=0.01
BKS = total of all breaks
EXC = total of all exchanges

Study outcome: Entecavir, at concentrations up to and including cytotoxic doses in both the absence and presence of S-9 metabolic activation, is clastogenic in the in vitro human

lymphocyte chromosome aberration assay. Thus, it is concluded that entecavir is clastogenic to cultured human lymphocytes.

Study title: 4. Entecavir: DNA repair study of entecavir in rats.

Key findings: Groups of male Sprague-Dawley rats (6/group) were orally gavaged with entecavir at single dose levels of 0 (control), 2 (low), 20 (mid), 200 (high) or 2000 mg/kg (very high). Three additional rats were given dimethylnitosamine orally at 10 mg/kg to serve as a positive control. Three treated animals and the positive control were euthanized at approximately 2 hr after dosing, and 3 treated animals and the vehicle controls were euthanized 16 hr after dosing to valuate the ability to induce unscheduled DNA synthesis (UDS). UDS evaluations for the treated rats, either 2 or 16 hr before the sacrifice, were clearly negative, as were those for the vehicle control groups. Conclusions: under the conditions of this assay, entecavir did not induce genotoxic damage that resulted in UDS in male rats.

Study no.: G037-96

Volume #, and page #: 1 and 1-43

Conducting laboratory and location: —

Date of study completion: November 6, 1996

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Batch # N005A. —

Methods

Entecavir was administered via oral gavage to groups of male Sprague-Dawley rats according to an experimental design shown in Table 52. Three additional rats were given dimethylnitosamine orally at 10 mg/kg to serve as a positive control. Three treated animals and the positive control were euthanized at approximately 2 hr after dosing, and 3 treated animals and the vehicle controls were euthanized 16 hr after dosing to valuate the ability to induce unscheduled DNA synthesis (UDS).

Table 52
Study design of unscheduled DNA synthesis (UDS) in rats

treatment	Dose (mg/kg)	Volume (ml/kg)	No. of animals dosed	
			2 hr	16 hr
Vehicle control	0	40	0	3
Entecavir	2	2	3	3
	20	20	3	3
	200	4	3	3
	2000	40	3	0
Dimethylnitrosamine, positive control	10	10	3	0

Strains/species/cell line: Table 52

Doses used in definitive study: Table 52

Basis of dose selection: dose levels were set by Bristol-Myers Squibb based on their experience from rat toxicology studies

Negative controls: Table 52

Positive controls: Table 52

Incubation and sampling times: following dosing, hepatocytes were obtained and were evaluated for unscheduled DNA synthesis (UDS).

Results:

Summary of UDS data are summarized in Table 53.

Table 53
Summary of unscheduled DNA synthesis assay in male Sprague-Dawley rats

Treatment	Dose (mg/kg)	Dosing interval (hr)	Nuclear	Cytoplasm	NG	%IR	NGIR
Vehicle control	0	16	3.5	7.3	-3.8	0	
Entecavir	2	2	3	5.5	-2.5	0	
		16	4.1	6.5	-2.4	0	
	20	2	3.9	6.9	-3	1	5.7
		16	3.6	6.3	-2.7	0	
	200	2	4.2	7.5	-3.3	0	
		16	3.1	5.3	-2.2	0	
	2000	2	4.8	8.2	-3.4	0	
		16	2.7	4.8	-2.1	0	
Dimethylnitrosamine, positive control	10	2	10.7	5.8	+4.9	41	11.4

Nuclear = nuclear grain count; the number of grain over the nucleus
Cytoplasm = cytoplasmic grain count; the highest grain count from 2 nuclear-sized areas adjacent to the nucleus

NG = net grain/nucleus; the nuclear count minus the cytoplasmic count
% IR (in repair) = percent of cells with at least 5 NG
NGIR = average net grains/nucleus of cells in repair

Study validity: valid

Study outcome: these results indicated that entecavir did not induce genotoxic damage that resulted in UDS in male rats hepatocytes after in vivo treatment.

Study title: 5. Entecavir: Oral micronucleus study in rats

Key findings: Groups of male rats (5 animals/group) were orally gavaged with entecavir at dose levels of 0 (control), 2 (low), 20 (mid), 200 (high) or 2000 mg/kg/day (very high) for 3 days to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. Due to unexpected drug related deaths at 2000 mg/kg dose level, a portion of the study was repeated at dose levels of 200 and 1500 mg/kg. Bone marrow cells were collected 24 hr after the treatment and were examined for micronucleated polychromatic erythrocytes (PCEs). Two animals died (high) following the third dose. Drug related clinical signs were observed in the high dose animals. Mild bone-marrow toxicity was apparent in the surviving high dose animals. The frequencies of PCEs in the bone marrow of treated rats were not significantly increased (p=0.05) in the treated groups when compared to the controls. Under the conditions of the test and according to the criteria set for evaluating the test results, entecavir was negative for in vivo genotoxic potential in the rat micronucleus assay.

Study no.: 96683

Volume #, and page #: 1 and 1-59

Conducting laboratory and location: **Bristol-Myers Squibb, New Brunswick, NJ**

Date of study completion: November 5, 1996

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: C009A, C011A and C012A; [redacted]

Methods

Groups of male rats (strain: [redacted] Sprague Dawley SD; age: 8 weeks; 5 animals/group) were orally gavaged with entecavir at dose levels of 0 (control), 2 (low), 20 (mid), 200 (high) or 2000 mg/kg/day (very high) for 3 days to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow according to an experimental design shown in Table 1. Due to unexpected drug related deaths at 2000 mg/kg dose level, a portion of the study was repeated at dose levels of 200 and 1500

mg/kg as shown in Table 54.

Table 54
Experimental design of entecavir oral micronucleus study in male rats

Group No.	Test article	Dose (mg/kg/day)	Dose volume (mg/kg)	No. of animals
1	Vehicle control	0	20	5 male
2	Entecavir	2	2	5 male
3		20	20	5 male
4		200	2	5 male
5		2000	20	5 male
6	Cyclophosphamide (positive)	7	10	5 male

Table 55
Experimental design for limited repeat of entecavir oral micronucleus study in male rats

Group No.	Test article	Dose (mg/kg/day)	Dose volume (mg/kg)	No. of animals
7	Vehicle control	0	30	5 male
8	Entecavir	200	4	5 male
9		1500	30	5 male
10	Cyclophosphamide (positive)	7	10	5 male

Strains/species/cell line: male rats, Hsd:Sprague Dawley SD

Doses used in definitive study: Tables 54 and 55

Basis of dose selection: a limited range finding study where entecavir was administered at a single dose level of 2000 mg/kg

Negative controls: Tables 54 and 55

Positive controls: Tables 54 and 55

Incubation and sampling times: approximately 24 hr after the last dose administration, femoral bone marrow was sampled for evaluation. Bone marrow toxic effects were evaluated based upon a determination of the percent polychromatic erythrocytes (PCEs or reticulocytes) of the total erythrocyte count.

Results

Clinical Signs and mortality: for the definitive and limited repeat studies are shown in Tables 56 and 57, respectively.

Table 56
Clinical signs and mortality during the definitive oral micronucleus in rats

Sex	Dose (mg/kg/day)	Incidence of death	Toxic signs
Male	Vehicle	0/5	No overt signs
Male	2	0/5	Soiled muzzle
Male	20	0/5	No overt signs
Male	200	0/5	No overt signs
Male	2000	2/5	Soiled muzzle, rough hair coat, diarrhea, decreased activity, soiled forepaws
male	Cyclophosphamide	0/5	No overt signs

Table 57
Clinical signs and mortality during the limited repeat oral micronucleus in rats

Sex	Dose (mg/kg/day)	Incidence of death	Toxic signs
Male	Vehicle	0/5	No overt signs
Male	200	0/5	No overt signs
Male	1500	0/5	Soiled muzzle, rough hair coat, diarrhea, decreased activity, soiled forepaws and hind paws. Soiled genitals, soiled eyes
male	Cyclophosphamide	0/5	No overt signs

Bone marrow PCEs in the definitive study: frequencies of PCEs in the definitive study are shown in Table 58. A mean frequency of 52% was observed in the vehicle controls. The frequencies of PCEs were 54%, 49%, 50% and 31% for the entecavir dose levels of 2, 20, 200 and 2000 mg/kg, respectively. Thus, mild bone marrow toxicity was observed at the top dose level (40% mean decrease in PCEs) compared to the vehicle controls. The frequencies of MN-PCEs in the bone marrow were 0.20%, 0.25%, 0.20%, 0.26% and 0.12% for the entecavir dose levels of 0, 2, 20, 200 and 2000 mg/kg, respectively; these frequencies were not statistically significant from the controls.

Table 58
Summary of bone marrow analysis for the definitive study

Test article	Dose (mg/kg/day)	No. of rats evaluated	Mean % PCEs	Mean % MN-PCEs
vehicle	Vehicle	5	52	0.20
entecavir	2	5	54	0.25
	20	5	49	0.20
	200	5	50	0.26
	2000	3	31	0.12
Cyclophosphamide, positive control	7	5	51	2.49

Bone marrow PCEs in the limited repeat study: frequencies of PCEs in the repeat study are shown in Table 59. A mean frequency of 53% was observed in the vehicle controls. The frequencies of PCEs were 57% and 38% for the entecavir dose levels of 200 and 1500 mg/kg, respectively. Thus, mild bone marrow toxicity was observed at the top dose level (28% mean decrease in PCEs) compared to the vehicle controls. The frequencies of MN-PCEs in the bone marrow were 0.16%, 0.17% and 0.33% for the entecavir dose levels of 0, 200 and 2000 mg/kg, respectively; these frequencies were not

statistically significant from the controls. The mild elevation in MN-PCEs at 1500 mg/kg dose was largely due to one animal; all individual animal values were within historical controls.

Table 59
Summary of bone marrow analysis for the limited repeat study

Test article	Dose (mg/kg/day)	No of rats evaluated	Mean % PCEs	Mean % MN-PCEs
Vehicle	0	5	53	0.16
Entecavir	200	5	57	0.17
	1500	5	38	0.33
Cyclophosphamide, positive control	7	5	50	1.93

Study validity: yes

Study outcome: Two animals died (high) following the third dose. Drug related clinical signs were observed in the high dose animals. Mild bone-marrow toxicity was apparent in the surviving high dose animals. The frequencies of PCEs in the bone marrow of treated rats were not significantly increased (p=0.05) in the treated groups when compared to the controls. Under the conditions of the test and according to the criteria set for evaluating the test results, entecavir was negative for in vivo genotoxic potential in the rat micronucleus assay.

2.6.6.5 Carcinogenicity (for detail see APPENDIX/ATTACHMENTS #1 (Exec CAC minutes and #2 Full CAC minutes)

Study title: 1. Entecavir: oral (gavage) oncogenicity study in rats

Key study findings: The oncogenicity potential of entecavir was investigated in male rats at oral gavage dosages of 0.003 (low), 0.02 (mid), 0.2 (high) or 1.4 mg/kg/day (highest) and in females at dose levels of 0.01 (low), 0.06 (mid), 0.4 (high) or 2.6 mg/kg/day (highest) in comparison with vehicle controls for a period of 96 (male) and 104 (female) weeks. The NOEL for neoplasia was 0.2 mg/kg/day for males and 0.06 mg/kg/day for females. At tumorigenic doses, systemic exposures were 35- and 4-times that in humans (1.0 mg daily dose) in male and female rats, respectively. The treatment induced hepatocellular adenomas in female rats were significant (p=0.005) at the highest dose level. Combined adenomas and carcinomas in the female rats were also significant (p=0.005) at the highest dose. In female rats, the combined incidence of adenomas and carcinomas was 1% (controls), 4% (low), 5% (mid), 2% (high) and 18% (highest). The exposure in the female rats (highest) was 24 times the exposure in humans. Brain gliomas were significant (p=0.025) at the highest dose in both male and female rats. In male rats, the incidence was 0% (controls), 2% (low), 2% (mid), 3% (high) and 7% (highest). In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 0% (high) and 5% (highest). The exposures in the male and female rats (highest) were 35 and 24 times the exposure in humans, respectively. Skin fibromas in female rats were significant (p=0.025) at the highest doses. In female rats, the incidence was 0% (controls), 0% (low),

2% (mid), 3% (high) and 5% (highest). The exposures in the female rats were 4 (high) and 24 (highest) time the exposure in humans.

With regard to non-neoplastic changes, hyperplastic changes were noted in the pancreas for males (high and highest). In addition, non proliferative findings were present in the liver for males and females (high and highest) and the testes and kidneys for males (highest). The NOELs for non-neoplastic changes were 0.02 mg/kg/day for males and 0.06 mg/kg/day for females.

Adequacy of the carcinogenicity study and appropriateness of the test model: yes

Evaluation of tumor findings:

1. Hepatocellular adenomas in female rats were significant (p=0.005) at the highest dose level. Combined adenomas and carcinomas in the female rats were also significant (p=0.005) at the highest dose. In female rats, the combined incidence of adenomas and carcinomas was 1% (controls), 4% (low), 5% (mid), 2% (high) and 18% (highest).

2. Brain gliomas were significant (p=0.025) at the highest dose in both male and female rats. In male rats, the incidence was 0% (controls), 2% (low), 2% (mid), 3% (high) and 7% (highest). In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 0% (high) and 5% (highest).

3. The skin fibromas in female rats were significant (p=0.025) at the highest doses. In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 3% (high) and 5% (highest).

Study no.: 99-2611/99024

Volume #, and page #: volumes 1-16, and pages 1-6281

Conducting laboratory and location: —

—

Date of study completion: 23 April, 1999

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Lot # **RO27B and RO27B(A), purity** —

CAC concurrence: no

Methods

Doses (mg/kg/day): Experimental design is shown in Table 60.

Table 60
Experimental design of rat carcinogenicity study

Dosage group (mg/kg/day		Carcinogenicity rats		Toxicokinetics rats		Termination	
						22 month	24 month
Male	Female	male	female	Male	female	male	Female
vehicle control 1	0	60	60	0	0	13	34
vehicle control 2	0	60	60	0	0	12	37
low; 0.003	0.01	60	60	10	10	14	30
mid; 0.02	0.06	60	60	10	10	15	32
high; 0.2	0.4	60	60	10	10	14	31
highest; 1.4	2.6	60	60	10	9	14	28

Basis of dose selection (MTD, MFD, AUC etc.): The doses selected for the study were based both on the results of a 6-month oral toxicology study in rats and on estimated steady-state systemic exposures (AUCs) in humans at 0.1 to 1.0 mg daily dose of entecavir.

Species/strain: Rat, (Outbred) —. Sprague-Dawley

Number/sex/group (main study): Table 60.

Route, formulation, volume: oral gavage, solution, 5 mg/kg

Frequency of dosing: once daily

Satellite groups used for toxicokinetics or special groups: Table 60

Age: 6 week

Animal housing: individual

Restriction paradigm for dietary restriction studies: none

Drug stability/homogeneity: adequate

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: not significant

Observation times

Mortality: once daily

Clinical signs: once daily

Body weights: once weekly for 13 weeks, twice weekly thereafter

Food consumption: once weekly for 13 weeks, twice weekly thereafter

Histopathology: Peer review: yes

Toxicokinetics: day 182

Results

Mortality: survivability at termination of the study is shown in Table 61. There were no statically significant effects of entecavir on mortality in any treatment group, as compared to the combined control groups. The percentage of animals dying prior to study termination and the times to death were similar in the drug treated groups. Among all males, including the controls, high mortality rates were observed; as a result, all male groups were terminated during week 94. The percentage of animals in each group surviving at that time point ranged from 20% to 25.4%. The leading cause of death in males in all groups was considered to be chronic progressive nephropathy. The percentage of females in all groups surviving 24 months ranged from 48.3% to 64.2%. Although mortality was slightly higher in the drug treated groups as compared to the controls, there was no dose relationship and there were no statistically significant differences between the controls and entecavir treated females.

Table 61
Survivability at termination of the rats oncogenicity study

Dosage Group (mg/kg/day)	Number of rats					
	Total dead		Total survivors		% survival	
	male	Female	Male	female	Male	Female
vehicle control 1	46	19	13	34	22	64.2
vehicle control 2	48	21	12	37	20	63.8
Low	42	28	14	30	25	51.7
mid	44	28	15	32	25.4	53.3
High	45	28	14	31	23.7	52.5
Highest	44	30	14	28	24.1	48.3

Clinical signs: findings observed during weekly physical examinations for all groups were generally the type commonly seen in laboratory rats and were not considered test

article related.

Body weights: data are shown in Table 62. Drug related decreases in mean body weight and body weight gain were noted for males (highest). Values for these parameters were decreased throughout the study and the decreases were statistically significant at many intervals, as compared to the control group 1. The decreases in mean body weight were minimal to mild, ranging from 1% to 9%. Prior to termination, mean body weight for the highest dose males was decreased 8% relative to the control value. For females (low, mid, high or highest), mean body weights and body weight gains were generally comparable to the control values. A few statistically significant increases in mean body weight and body weight gain were noted sporadically and were not considered drug related.

Table 62
Group mean body weights (g) during the rats oncogenicity study

Dosage Group (mg/kg/day)	group mean body weights (g)							
	week 1		week 35		week 71		Week 93/103	
	male	Female	male	Female	Male	female	Male	Female
Vehicle control 1	273	193	548	291	581	304	534	326
Vehicle control 2	273	192	546	293	578	313	559	315
(low)	273	192	559	294	586	323	587	342
(mid)	272	193	547	292	580	317	550	323
(high)	268	192	538	291	561	320	523	319
(highest)	268	190	531	285	541**	313	491	309

** = $p<0.01$

Food consumption: values are shown in Table 63. Statistically significant drug related increases in food consumption were noted at various intervals in the study for males and females, as compared to the control group 1. Other statistically significant increases in food consumption were noted sporadically during the study for the other drug groups, as well as the control 2 group.

Table 63
Group mean food consumption (g/day) during the rats oncogenicity study

Dosage Group (mg/kg/day)	Group mean food consumption values (g/kg/day)							
	week 1		week 35		week 71		Week 93/103	
	male	Female	male	Female	Male	female	Male	Female
Vehicle control 1	91	91	44	56	42	55	42	55
Vehicle control 2	91	92	44	59**	41	55	40	52
(low)	95**	97**	45	60**	42	56	40	56
(mid)	95**	95**	47**	63**	44**	57	39	52
(high)	93**	95**	45	60**	42	56	43	54
(highest)	95**	96**	48**	64**	46**	60**	43	57

** = $p<0.01$

Gross pathology:

LIVER: In males and females, increased incidences of discolored livers were observed (high and highest). In a number of these animals, the macroscopic discolorations correlated with microscopic findings of hepatocellular alterations and/or hepatocellular vacuolation. The most frequently noted discoloration was pallor of the liver; this was followed by variable incidences of brown/tan foci, red/dark/black foci, and white foci.

KIDNEYS: In males, the incidences of kidneys with irregular surfaces were increased (high and highest). The incidence of renal cysts was also increased in males at the highest dose level. Macroscopic findings of irregular surfaces correlated with the microscopic findings of chronic progressive nephropathy. Cysts were also seen microscopically in a number of animals.

LIVER: Numerous males and females in all groups, including the controls, had altered hepatocellular foci/areas(basophilic, eosinophilic and occasionally clear cell); overall, severity ranged from minimal to moderate (Table 64). In both sexes, the incidence and severity of basophilic and eosinophilic foci/areas were greatest at the highest dose level. In both males and females, the incidence of clear cell foci/areas in all of the drug treated groups was comparable to the controls.

Table 64
Non-proliferative findings in rats administered entecavir

Group	Dosages											
	Control 1 (0)		control 2 (0)		low		mid		high		Highest	
Sex	male	female	male	female	male	female	male	female	male	female	male	female
Hepatocellular alterations:												
Basophilic	6/60	12/60	3/60	10/60	3/59	13/57	1/60	19/60	2/60	31/60	11/60	33/60
Eosinophilic	16/60	6/60	8/60	10/60	11/59	8/57	9/60	13/60	18/60	18/60	35/60	30/60
Clear cell	2/60	1/60	0/60	2/60	1/59	1/57	1/60	2/60	0/60	8/60	3/60	4/60
Hepatocytes vacuolated	8/60	3/60	8/60	2/60	10/59	4/57	9/60	6/60	10/60	6/60	24/60	15/60
Reticuloendothelial cells: brown pigment	3/60	1/60	1/60	9/60	2/59	4/57	0/60	0/60	0/60	10/60	2/60	19/60

TESTES: Numerous males in all groups had maturation arrest/degeneration/atrophy of the germinal epithelium (minimum to severe); the incidence was greatest at the highest dose level. In conjunction with this testicular change, incidences of oligospermia and intraluminal degenerated seminal product (overall minimal to severe) in the epididymides and decreased secretion in the prostate gland, seminal vesicles and coagulating gland (overall slight to marked) were also greatest at the highest dose level.

KIDNEYS: Almost all males and numerous females in the control and drug treated group had chronic progressive nephropathy (minimal to marked). Scattered convoluted tubules with hypertrophic epithelium (minimal to moderate) occurred in numerous males in all groups and occurred sporadically in females. In the males this hypertrophic change was considered to be a sequela to the chronic progressive nephropathy; the incidence and severity of both findings were greatest at the highest dose level. In addition, the leading cause of death in males in all groups was considered to be chronic progressive nephropathy.

Neoplastic: Statistical significant increased incidences of proliferative lesions were observed in the pancreas of males (high and highest), in the brains of males and females (highest) and in livers of females (highest).

PANCREAS: Focal hyperplasia of the acinar (exocrine) pancreas was present in a number of males in all groups (Table 65), including the controls; the incidence was comparable at the high and highest dose levels and for both groups was greater than the control and other treated groups. Foci of hyperplasia were multiple in one rat (low and mid). There is a histologic continuum between focal acinar (exocrine) cell hyperplasia and acinar (exocrine) cell adenoma. In this study, the well-differentiated proliferative acinar cell lesions approximately 5 mm in diameter or greater were classified as adenomas. Four males had an acinar cell adenomas of the pancreas: two each at the high

and highest dose levels. The incidence of the adenomas was not statistically significant using the Peto analysis.

Table 65

Proliferative lesions of the exocrine pancreas: male rats administered entecavir

Lesions	Dosage levels (mg/kg/day)					
	control 1 (0)	control 2	low (0.003)	mid (0.02)	high (0.2)	Highest (1.4)
Hyperplasia	7/59 (11.9%)	7/58 (12.1%)	5/59 (8.5%)	10/59 (16.9%)	28/60 (46.7%)	24/60 (40%)
ADENOMA	0/59	0/58	0/59	0/59	2/60 (3.3%)	2/60 (3.3%)
Carcinoma	0/59	0/58	0/59	0/59	0/60	1/60 (1.7%)
Mixed islet-acinar cell neoplasm	0/59	0/58	0/59	0/59	0-60	1/60 (1.7%)

BRAIN: Four males and three females (highest) had malignant gliomas (Table 66). One additional female (highest) had a malignant oligodendroglioma. In males and females (highest), the incidences were statistically significant using the Peto analysis ($p < 0.025$).

Table 66

Brain malignant gliomas in rats administered entecavir

Group; dosage (mg/kg/day)	Malignant gliomas of brain in rats	
	Male	Female
Control 1	0/60	0/60
Control 2	0/60	0/60
Low	1/60 (1.7%)	0/60
Mid	1/60 (1.7%)	1/60 (1.7%)
High	2/60 (3.3%)	0/60
Highest	4/60 (6.7%)	3/60 (5%*)

* = statistically significant using the Peto analysis ($p < 0.025$)

LIVER: Fifteen females had hepatocellular adenoma and three females had hepatocellular carcinoma; the distribution of these neoplasms is presented in Table 67. The incidences of both neoplasms were greatest in the highest dose females. The incidences of hepatocellular adenoma and carcinoma in the highest dose females were statistically significant using the Peto analysis ($p < 0.005$).

Table 67
Neoplasms in the liver of female rats administered entecavir

Group; dosage (mg/kg/day)	Neoplasms of liver in female rats	
	Adenoma	***CARCINOMA***
Control 1	0/60	0/60
Control 2	1/60 (1.7%)	0/60
Low; 0.01	2/57 (3.5%)	0/57
mid; 0.06	3/60 (5%)	0/60
High; 0.4	1/60 (1.7%)	0/60
Highest; 2.6	8/60 (13.3%)	3/60 (5%)

OTHER NEOPLASMS: in male and female rats are shown in Tables 68 and 69.

Table 68
Other neoplasms in female rats administered entecavir

Group; dosage (mg/kg/day)	Other neoplasms in female rats		
	Zymbal's gland: squamous/squamo-sebaceous cell carcinoma	Uterus: hemangiosarcoma	Skin: fibroma
Control 1	0/58	0/60	0/60
Control 2	0/59	0/60	0/60
Low; 0.01	0/60	0/60	0/60
Mid; 0.06	0/60	0/59	1/60
High; 0.4	1/60	0/59	2/57 (3%)
Highest; 2.6	3/60 (5%)	2/60 (3%)	3/60 (5%)

Table 69
Other neoplasms in male rats administered entecavir

Group; dosage (mg/kg/day)	Other neoplasms in male rats: kidneys	
	Oncocytoma	malignant mesenchymal tumor
Control 1	0/60	0/60
Control 2	0/60	0/60
Low; 0.003	0/60	0/60
mid; 0.02	0/60	0/60
High; 0.2	0/60	0/60
Highest; 1.4	2/60 (3%)	2/60 (3%)

Summary of rat neoplasms: is shown in Table 70.

Table 70

Incidence (%) of rat neoplasms in entecavir carcinogenicity studies. Multiple of exposure relative to a 1.0 mg dose in humans is shown in parentheses

Neoplasm	Sex	0	Low	mid	high	Highest	Historical control range
Brain glioma	Male	0	2%(0)	2%(0.3)	3%(5)	7%*(35)	0-3%
Pancreas adenoma	Male	0	0	0	3	3%	Na
Pancreas carcinoma	Male	0	0	0	0	2%	Na
COMBINED	Male	0	0	0	3%	5%	
Brain glioma	Female	0	0(0.4)	2%(1)	0(4)	5%*(24)	0-1.5%
Liver adenoma	Female	1%	4%	5%	2%	13%**	0-5%
Liver carcinoma	Female	0	0	0	0	5%	0%
COMBINED	Female	1%	4%	5%	2%	18%**	
Skin fibroma	Female	0	0	2%	3%	5%*	0-7%

* = statistically significant for rare tumors at $p < 0.025$
** = statistically-significant tumors determined using Peto analysis for common tumors at $p < 0.005$
d = despite high incidence, not significant using Peto analysis for common tumor at $p < 0.005$
na = not available

Toxicokinetics: mean plasma concentrations are shown in Table 71 for male and female rats, respectively. These data suggest that the systemic exposures of the rats to entecavir were dose related following the administration in the study.

Table 71

Entecavir mean plasma concentrations (ng/ml) in the oncogenicity study in male rats

Group; dosage (mg/kg/day)	Entecavir mean plasma concentrations (ng/ml)	
	day 182	
	1 hr	4 hr
0.003 (low)	0.17	Nd
0.02 (mid)	1.36	0.51
0.2 (high)	22.17	5.55
1.4 (highest)	221.01	53.79

nd= not detected

AUC values from the following mice study were used for comparing exposures (15 and 26.6 ng*hr/ml, respectively) at the clinical daily doses of 0.5 mg and 1.0 mg in humans.

Entecavir: One-month oral toxicokinetic study in rats (Study No. 92000376/DM00013)

Groups of male and female rats (12 animals/group) received repeated oral doses of entecavir at dose levels of 0.003, 0.02, 0.2, or 1.4 mg/kg/day in males and 0.01, 0.06, 0.4 or 2.6 mg/kg/day in females for 28 consecutive days. Blood samples for toxicokinetic investigations were collected at 0.5, 1, 2, 3, 4, 6, 8 and 24 hr post dosing on day 28 of the study. Plasma concentrations of entecavir were determined by a validated LC/MS/MS method. Results: are shown in Table 72. The systemic exposure (AUC and Cmax) to entecavir in females was dose related.

Table 72

Summary of mean toxicokinetic parameters in a 4-week toxicokinetic study in rats

Dosage (mg/kg/day)		AUC_{24} (ng*hr/ml)	Cmax (ng/ml)
0.003	Male	-	-
0.02		9.2	1.2
0.2		124	20.4
1.4		928	175
0.01	Female	9.7	1.2
0.06		18	2.6
0.4		116	44.5
2.6		636	193

Study title: 2. Entecavir: oral (gavage) oncogenicity study in mice

Key study findings: : The oncogenicity potential of entecavir was investigated in mice at oral gavage dosages of 0.004 (low), 0.04 (mid), 0.4 (high) or 4.0 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The NOEL for neoplasia was 0.004 mg/kg/day for males, based on pulmonary adenomas; for all other tumors in males and females, the NOEL was 0.4 mg/kg/day. At the tumorigenic dose in male mice, systemic exposure was 3-times that in humans (1.0 mg daily dose). The treatment induced bronchioloalveolar adenomas in male mice achieved statistical significance (p=0.005; mid, high and highest) and increased in a dose related manner at exposures multiples of 3 and higher relative to the daily clinical dose of 1.0 mg. In male mice, combined lung adenomas and carcinomas also achieved statistical significance (p=0.005) increased in the dose related manner at exposures multiples of 3 and higher relative to the daily clinical dose of 1.0 mg. In male mice, the combined incidence of adenomas and carcinomas was 12% (controls), 20% (low), 26% (mid), 40% (high) and 58% (highest). In female mice, the combined incidence of adenomas and carcinomas was 20%

(controls), 13% (low), 10% (mid), 35% (high) and 52% (highest). Hepatocellular carcinomas in the male mice were significant (p=0.005) at the highest dose level. The exposure in the male mice was 42 times the exposure in humans. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant (p=0.005) at the highest dose level. In female mice, the incidence of all vascular tumors was 16% (controls), 23% (low), 29% (mid), 26% (high) and 64% (highest). The exposure in the female mice was 40 times (highest) the exposure in humans.

Non-neoplastic changes noted after the administration of entecavir to mice included inflammatory changes in the lungs for males and females (high and highest) and ovarian lesions for females (highest). The NOEL for non-neoplastic changes was 0.04 mg/kg/day for males and females (3-time the exposure in humans).

Adequacy of the carcinogenicity study and appropriateness of the test model: yes

Evaluation of tumor findings:

1. Lung adenomas were significant (p=0.005) in male mice (mid, high and highest) and in the female mice at the highest dose (p=0.005); lung carcinomas in both male and female mice were significant (p=0.005) at the highest dose. Combined lung adenomas and carcinomas were significant (p=0.005) in male mice at the mid, high and highest dose levels and in the female at the highest dose level (p=0.005. In male mice, the combined incidence of adenomas and carcinomas was 12% (controls), 20% (low), 26% (mid), 40% (high) and 58% (highest). In female mice, the combined incidence of adenomas and carcinomas was 20% (controls), 13% (low), 10% (mid), 35% (high) and 52% (highest).

2. Hepatocellular carcinomas in male mice were significant (p=0.005) at the highest dose level. Combined liver adenomas and carcinomas were also significant (p=0.005) at the highest dose level in the male mice. In male mice, the combined incidence of adenomas and carcinomas was 11% (controls), 9% (low), 8% (mid), 16% (high) and 25% (highest).

3. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant (p=0.005) at the highest dose level. In female mice, the incidence of vascular tumors was 16% (controls), 23% (low), 29% (mid), 26% (high) and 64% (highest).

Study no.: (99-2612/99025)

Volume #, and page #: Volumes 1-10, pages 1-4281

Conducting laboratory and location:

Date of study completion: 4 June 1999

GLP compliance: Yes

QA report: yes

Drug, lot #, and % purity: lot # **RO27B and RO27B(A), purity**

CAC concurrence: No

Methods

Doses: are shown in Table 73

Table 73
Experimental design of the oral (gavage) 24-month oncogenicity study in mice

Dosage group (mg/kg/day	Carcinogenicity mice		Toxicokinetics mice		Necropsy termination	
	male	Female	male	female	Male	female
vehicle control 1	60	60	0	0	27	24
vehicle control 2	60	60	0	0	23	31
low; 0.004	60	60	8	10	24	21
mid; 0.04	60	60	10	10	20	23
high; 0.4	60	60	10	8	22	17
highest; 4.0	60	60	10	8	10	8

Basis of dose selection (MTD, MFD, AUC etc.): The doses selected for the study were based both on the results of a 6-month oral toxicology study in mice and on estimated steady-state systemic exposures (AUCs) in humans at 0.1 to 1.0 mg daily dose of entecavir.

Species/strain: albino mice (Outbred) VAF/Plus CD-1 BR

Number/sex/group (main study): Table 73

Route, formulation, volume: oral gavage, solution, 5 ml/kg

Frequency of dosing: once daily

Satellite groups used for toxicokinetics or special groups: Table 73

Age: 46 days

Animal housing: individual

Restriction paradigm for dietary restriction studies: none

Drug stability/homogeneity: adequate

Dual controls employed: yes

Interim sacrifices: no

Deviations from original study protocol: not significant

Observation times

Mortality: once daily

Clinical signs: once daily

Body weights: once weekly for 13 weeks, twice weekly thereafter

Food consumption: once weekly for 13 weeks, twice weekly thereafter

Histopathology: Peer review: yes

Toxicokinetics: day 182

Results

Mortality: survivability at termination of the study is shown in Table 74. There were drug related effects on mortality for males (highest) and females (high and highest). The percentage of animals surviving to study termination was decreased for these groups compared to the control groups. A significant number of deaths in males and females (highest) were attributed to severe lung lesions and lung tumors. In addition, vascular tumors, mostly of ovarian origin, were considered to be the cause of death for a number of females in the highest dose group.

Table 74
Survivability at termination of the mice oncogenicity study

Dosage Group (mg/kg/day)	Number of mice					
	Total dead		Total survivors		% survival	
	male	Female	male	female	male	female
Vehicle control 1	32	34	27	24	45.8	41.4
Vehicle control 2	37	27	23	32	38.3	54.2
low; 0.004	34	38	24	21	41.4	35.6
mid; 0.04	39	36	20	23	33.9	39
high; 0.4	37	41	22	17	37.3	29.3
highest; 4.0	50	51	10	8	16.7	13.6

Clinical signs: findings observed during weekly physical examinations for all groups were generally the type commonly seen in laboratory mice and were not considered test article related.

Body weights: data are shown in Table 75. Drug related decreases in mean body weights and body weight gains were noted for males and females at the highest dose level. Values for these parameters were generally decreased throughout the study and the decreases were statistically significant at many intervals, as compared to the control group 1. The decreases in mean body weight were minimal to mild, ranging from 1% to 12% in males and 1% to 18% in females. Prior to study termination, mean body weights for males and females (highest) were decreased 11% and 16%, respectively, as compared to the control values.

For males and females (low, mid or high), mean body weights and body weight gains were generally comparable to the control values.

Table 75
Group mean body weights (g) during the mice oncogenicity study

Dosage Group (mg/kg/day)	group mean body weights (g)							
	week 1		week 35		week 71		week 104	
	male	Female	male	female	male	female	Male	female
Vehicle control 1	27.6	22.5	37	29.8	36.4	30.6	34.7	30.4
Vehicle control 2	27	22.3	36.9	30	36.9	32.1	36.5	31
0.004 (low)	26.8	22.7	36.3	30.5	36.7	31.9	35.2	30
0.04 (mid)	27.3	22.6	36.9	29.8	36.4	31	35.4	30.6
0.4 (high)	26.7	22.6	35.3*	29.2	34.9	29.4	33.3	29.7
4 (highest)	28.3	22.4	34.8**	28.7	32.5**	28.9*	30.8*	25.7**

* = $p<0.05$
** = $p<0.01$

Food consumption: values are shown in Table 76. Statistically significant drug related increases in food consumption were noted at most intervals in the study for males (mid, high and highest) and females (highest), as compared to the control group 1. Other statistically significant increases in food consumption were noted sporadically during the study for the other drug groups, as well as the control 2 group.

Table 76

Group mean food consumption (g/day) during the mice oncogenicity study

Dosage Group (mg/kg/day)	Group mean food consumption values (g/kg/day)							
	week 1		week 35		week 71		week 104	
	male	Female	male	female	male	female	Male	female
Vehicle control 1	171.5	205.1	132	161.8	133.5	148.9	126	153.1
Vehicle control 2	187.6*	203.1	140.5*	169.5	142.3	149.5	127.5	152.1
0.004 (low)	193.3**	210.1	143.2**	178.4**	129.3	149.5	136.6	144.9
0.04 (mid)	192.1**	223.7	151.3**	183.1**	139.1	154.6	128.9	156
0.4 (high)	201.1**	208.3	159.7**	190.4**	145.4**	161.1**	141.8*	156.3
4 (highest)	199**	229.9*	167.8**	189.8**	154.2**	178.6**	149.2*	167.1

* = $p<0.05$
** = $p<0.01$

Gross pathology: observations that were related to treatment included increased incidences of liver masses and lung nodules in males (highest). These observations correlated with increased incidences of hepatocellular tumors in the liver and bronchiolo/alveolar tumors in the lungs. The lungs from both sexes of animals (highest) had a high incidence of lung discoloration, which correlated with the various inflammatory changes seen in these animals. In addition, salivary gland masses in two females (highest) correlated with tumors in this tissue.

LUNGS: The main findings are shown in Table 77. The findings were mostly inflammatory in nature that were drug related included leukocytosis/interstitial inflammation, infiltration of the alveolar spaces by alveolar macrophages and alveolar foamy macrophages, and alveolar fibrosis (highest dose only). The alveolar fibrosis appeared to be largely secondary to the interstitial inflammation. Severe inflammatory lung lesions (lung disease) were responsible for a significant number of deaths in males and females (highest).

Table 77

Selective inflammatory changes in the lungs of mice administered entecavir

Group	Dosage levels (mg/kg/day)											
	control 1 (0)		Control 2 (0)		low (0.004)		mid (0.04)		high (0.4)		Highest (4)	
Sex	male	female	male	female	male	female	male	female	male	female	male	female
Leukocytosis/interstitial inflammatory infiltrate	10	15	13	11	10	21	20	16	30	29	47	50
Alveolar macrophages	13	14	20	17	10	14	12	13	30	35	54	56
Alveolar foamy macrophages	0	2	4	2	3	0	1	2	8	8	48	41
Alveolar fibrosis	5	5	6	3	2	1	2	2	0	0	15	19
No. of lungs examined	60	60	60	60	60	60	58	60	60	60	60	60

OVARIES AND FEMALE REPRODUCTIVE TRACT: Compared with the controls, the incidences of hematocysts, thrombi and vascular ectasia in the ovaries of females (highest) were increased. Histologically, these lesions appeared to form a continuum with benign hemangiomas, the incidence of which was also increased (highest).

Atrophic changes in the uterus and vagina appeared to be increased in incidence in the highest dose females. In the uterus, these findings included an increase in the incidence of contracted/inactive uteri and a decrease in the incidence and severity of cystic endometrial hyperplasia/adenomyosis. In the vagina, there was an increase in the incidence of atrophic epithelium as well as a decrease in the incidence of animals showing squamous-cell hyperplasia of the vaginal epithelium.

LYMPH NODES: In comparison with controls, the mediastinal lymph nodes of males (highest) showed increased incidences and severity of lymphoid-cell hyperplasia and plasma cell hyperplasia. The females from this group also showed slightly increased severity of plasma-cell hyperplasia. Both findings are increased severity of plasma-cell hyperplasia.

Neoplastic: Statistical significantly increased incidences of proliferative lesions included bronchiolo/alveolar adenomas in males (mid, high and highest) and females (highest) and bronchiolo/alveolar carcinomas in males and females (highest), hepatocellular carcinomas in males (highest), and ovarian and uterine hemangiomas, splenic hemangiosarcomas, and salivary gland adenoacanthomas in females (highest).

LUNGS: Table 78 shows the incidence of proliferative lesions in the lungs of mice administered entecavir. The incidence of proliferative lesions including bronchiolo/alveolar adenomas and/or carcinomas and/or focal alveolar or bronchiolar epithelial hyperplasia showed dose-related increases in males at doses > 0.04 mg/kg/day and in females at doses > 0.4 mg/kg/day. The incidence of bronchiolo/alveolar adenomas and total combined adenomas and carcinomas were statistically significant by Peto

analysis at mid, high and highest dose levels for males and at highest dose level for females. The incidences of bronchiolo/alveolar carcinomas were statistically significant for both males and females (highest). [Since focal hyperplasia of the alveolar and bronchiolar epithelium forms a morphological continuum with bronchiolo-alveolar adenoma, they have also been included in the table 78 to provide a total incidence of proliferative lesions of the alveolar and bronchiolar epithelium]. In both males and females, there were increased incidences of focal hyperplasia of alveolar epithelium (high and highest) and focal hyperplasia of bronchiolar epithelium at the highest dose. Lung tumors were responsible for a significant number of deaths in both sexes (highest).

Table 78
Proliferative lesions in the lungs of mice administered entecavir

Proliferative lesions	Dosage levels (mg/kg/day)											
	control 1 (0)		Control 2 (0)		low (0.004)		mid (0.04)		high (0.4)		Highest (4)	
	male	female	male	Female	male	female	male	female	male	female	male	female
Bronchiolo/alveolar adenoma	4	9	4	6	8	5	11a	4	17a	16	20a	15a
Bronchiolo/alveolar carcinoma	3	3	3	5	4	3	4	2	7	5	15a	16a
All bronchiolo/alveolar tumors	7	12	7	11	12	8	15a	6	24ab	21b	35ab	31ab
Focal alveolar epithelium: hyperplasia	3	0	4	1	2	1	1	4	8	11	21	26
Focal bronchiolar epithelium hyperplasia	1	2	0	0	0	0	1	0	3	5	16	16
Total proliferative lesions	11	14	11	12	14	9	17	10	45	37	72	73
No. of lungs examined	60	60	60	60	60	60	58	60	60	60	60	60

a: denotes statistical significance by Peto analysis (p-value less than 0.005 for a common tumor or 0.025 for a rare tumor)
b: a few animals in this group had multiple lung tumors and were counted only once for Peto analysis

VASCULAR TUMORS: Compared with the control groups there were high incidences of vascular tumors (hemangioma) in the ovaries and uterus of females at the highest dose level (Table 79).

The incidence of vascular tumors in the body as a whole was also analyzed. The incidences of disseminated vascular tumors (at least one hemangiosarcoma in any tissue), localized vascular tumors (at least one hemangioma in any tissue) and all vascular tumors (total hemangiomas plus hemangiosarcomas in all tissues) were all statistically significant by the Peto analysis in females from the highest dose group. Vascular tumors, mostly of ovarian origin, were considered to be the cause of death for a number of females (highest).

Table 79

Incidence of statistically significant primary vascular tumors in the ovary, uterus and spleen of female mice administered entecavir

Organs	Dosage levels (mg/kg/day)					
	control 1 (0)	Control 2	low (0.004)	mid (0.04)	high (0.4)	highest (4)
Ovaries:						
Hemangioma	3 (5%)	3 (5%)	6 (10%)	7 (12%)	5 (8%)	19 (32%)
No. of ovaries examined	60	58	59	60	60	60
Uterus:						
Hemangioma	4(7%)	3 (5%)	4 (7%)	4 (7%)	5 (8%)	8 (13%)
No. of uterus examined	60	60	60	60	60	60
Spleen:						
Hemangiosarcoma	0	1 (2%)	0	2 (3%)	2 (3%)	2 (3%)
No. of spleen examined	60	59	60	60	60	60

LIVER: Table 80 summarized the incidence of hepatocellular tumors. The incidences of hepatocellular carcinomas and combined hepatocellular adenomas and carcinomas were statistically significantly increased in males (highest). The incidence of hepatocellular adenomas was similar between control and treated groups. The increased tumor incidence in the males was not accompanied by an increase in foci of hepatocellular alteration (basophilic, eosinophilic or clear cell).

Table 80

Incidence of hepatocellular tumors in the livers of mice administered entecavir

Group	Dosage levels (mg/kg/day)											
	Control 1 (0)		control 2 (0)		low (0.004)		mid (0.04)		high (0.4)		highest (4)	
Sex	male	female	male	female	male	female	male	female	male	female	male	female
Hepatocellular adenoma	8	0	4	2	4	0	2	1	8	0	7	2
Hepatocellular carcinoma	1	0	0	0	1	0	3	1	2	0	8a	0
Total hepatocellular tumors	9	0	4	2	5	0	5	2	10b	0	15ab	2
no. of livers examined	60	60	60	60	60	60	60	60	60	60	60	60

a: denotes statistical significance by Peto analysis (p-value less than 0.005 for a common tumor or 0.025 for a rare tumor)

b: a few animals in this group had multiple lung tumors and were counted only once for Peto analysis

SALIVARY GLANDS: Two similar but unusual tumors of the salivary gland were seen

in females from the highest dose level. Compared with zero incidences in the controls, the incidence was significant by Peto analysis ($p < 0.025$). The tumors appeared to originate from the parotid salivary gland and have been diagnosed as ductal adenoacanthoma. These tumors have not been described as spontaneous tumors in mouse.

Summary of mice neoplasms: is shown in Table 81.

Table 81

Incidence (%) of certain neoplasms in mice entecavir carcinogenicity studies. Multiple of exposure relative to a 1.0 mg dose in humans is shown in parentheses

Neoplasm	Sex	0	Low	mid	high	Highest	Historical control range
Lung adenomas	Male	7%	13%(1)	19%**(3)	28%**(13)	33%**(42)	6-31%
Lung carcinoma	Male	5%	7%	7%	12%	25%**	0-14%
Combined	Male	12%	20%	26%**	40%**	58%**	
Liver adenoma	Male	10%	7%	3%	13%	12%	na
Liver carcinoma	Male	1%	2%	5%	3%	13%**	0-16%
COMBINED	Male	11%	9%	8%	16%	25%**	
Lung adenomas	Female	13%	8%(1)	7%(3)	27%d(10)	25%**(40)	3-16%
Lung carcinoma	Female	7%	5%	3%	8%	27%**	2-14%
Combined	Female	20%	13%	10%	35%	52%**	
Liver adenomas	Female	0	3%	0	2%	3%	na
Liver carcinomas	Female	0	0	2%	0	0%	Na
COMBINED	Female	0	3%	2%	2%	3%	
Ovaries: Hemangioma:	Female	5%	10%	12%	8%	32%**	0-7%
Uterus: Hemangioma:		6%	7%	7%	8%	13%**	0-6%
Spleen: Hemangiosarcoma		1%	0	3%	3%	3%	1-2%
Hemangioma/hemangiosarcoma		4%	6%	7%	7%	16%**	0-7%

* = statistically significant for rare tumors at $p < 0.025$

** = statistically-significant tumors determined using Peto analysis for common tumors at $p < 0.005$

d = despite high incidence, not significant using Peto analysis for common tumor at $p < 0.005$

na = not available

Toxicokinetics:

Mean plasma concentrations are shown in Table 82. The plasma concentrations of entecavir appeared to be similar in male and female mice and increased as the daily dose

was increased. These data suggest that the systemic exposures of the mice to entecavir were dose related following the administration in the study.

Table 82
Entecavir mean plasma concentrations (ng/ml) in the oncogenicity study in mice

Group; dosage (mg/kg/day)		entecavir mean plasma concentrations (ng/ml)	
		day 182	
		1 hr	4 hr
0.004 (low)	male	0.49	0.45
	female	0.57	1.19
0.04 (mid)	male	2.15	1.83
	female	2.41	1.49
0.4 (high)	male	22.56	4.52
	female	32.53	5.86
4 (highest)	male	179.79	15.46
	female	290.84	11.46

AUC values from the following mice study were used for comparing exposures (15 and 26.6 ng*hr/ml) at the clinical daily doses of 0.5 mg and 10 mg in humans.

Entecavir: One-month oral toxicokinetic study in mice (Study No. 92000376/DM00013)

Groups of male and female mice (26 animals/sex/group) received repeated oral doses of entecavir at dose levels of 0.004, 0.04, 0.4, or 4 mg/kg/day for 28 consecutive days. Blood samples for toxicokinetic investigations were collected at 0.5, 0.75, 1, 2, 3, 4, 8, 12 and 24 hr post dosing on day 28 of the study. Plasma concentrations of entecavir were determined by a validated LC/MS/MS method. Results: are shown in Table 83. The systemic exposure (AUC and Cmax) to entecavir in mice increased in a less than dose proportional manner. The systemic exposures to entecavir were similar in male and female mice.

Table 83
Summary of mean toxicokinetic parameters in a 4-week toxicokinetic study in mice

Dosage (mg/kg/day)	AUC_{24} (ng*hr/ml)		Cmax (ng/ml)	
	Male	female	male	Female
0.004; low	27.3	29.8	2.7	2.3
0.04; mid	76.4	75.1	8.7	7.6
0.4; high	336	279	52.9	67.6
4; highest	1118	1053	440	515

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: 1. Entecavir: Oral study of fertility and early embryonic development in female rats

Key study findings: female rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) once daily for approximately 2 weeks. There were no drug related changes at any dose tested. Entecavir had no effect on reproductive function in females (estrous cycling, mating and fertility) or on the early embryonic development of the offsprings at any dose level. In this study, a dose level of 30 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent oral dose in humans would be 4.87 mg/kg/day (292 mg/day for a 60 kg person).

Study no.: 920008323/DN00006

Volume # and page #: 1 and 1-83

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: 15 November, 2000

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: RO23B, —

Methods

Doses: 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high)

Species/strain: virgin female rats (~.CD(SD)IGS BR,

Number/sex/group: 25 rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: female rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) once daily for approximately 2 weeks. Following the dosing, the female rats were placed in cohabitation with vehicle male rats for a maximum of 3 weeks. Daily dosing continued until day 7 of gestation. On day 16 of gestation, the dams and conceptuses were sacrificed and evaluated.
Parameters and endpoints evaluated: estrous cycling, mating or fertility

Results

Mortality: no drug related changes at any dose tested

Clinical signs: no effect

Body weight: no effect

Food consumption: no effect

Toxicokinetics: not done

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): entecavir had no effect on reproductive function in females (estrous cycling, mating and fertility) or on the early embryonic development of the offsprings at any dose level.

Study title: 2. Entecavir: Oral study of fertility and early embryonic development in male rats

Key study findings: male rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.1 (low), 1 (mid) or 10 mg/kg/day (high) once daily for approximately 4 weeks. Entecavir had no effect on fertility, mating or early embryonic development of the offsprings at any dose level. Similarly, reproductive organs weights (testes, epididymides and prostate/seminal vesicles) and sperm parameters (motility, morphology and counts) were unaffected by the treatment. In this study, a dose level of 1 mg/kg/day may be considered the NOEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.16 mg/kg/day (9.7 mg/day for a 60 kg person). Entecavir had no effect on reproductive function in male rats. A dose level of 10 mg/kg/day may be considered the NOEL for male reproductive toxicity. Based on the body surface area factor, an equivalent oral dose in humans would be 1.6 mg/kg/day (97.4 mg/day for a 60 kg person).

Study no.: 920008324/DN00014

Volume #, and page #: 1 and 1-113

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: 15 September 2000

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: # RO23B, —

Methods

Doses: 0 (sterile water, vehicle control), 0.1 (low), 1 (mid) or 10 mg/kg/day (high)

Species/strain: male rats —.CD(SD)IGS BR

Number/sex/group: 25 rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: male rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.1 (low), 1 (mid) or 10 mg/kg/day (high) once daily for approximately 4 weeks. Following the dosing, the male rats were placed in cohabitation with vehicle female rats for a maximum of 2 weeks. Daily dosing continued until scheduled termination (33 to 42 daily doses). The untreated females were sacrificed and cesarean-sectioned on day 16 of presumed gestation.

Parameters and endpoints evaluated: effect on fertility, mating or early embryonic development of the offsprings, reproductive organs and sperm parameters (motility, morphology and counts.

Results

Mortality: none

Clinical signs: no drug related changes

Body weight: there were no drug related changes occurred in animals (low and mid). At the high dose, decreased body weight and body weight gain were noted.

Food consumption: no changes

Toxicokinetics: not done

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Entecavir had no effect on fertility, mating or early embryonic development of the offsprings at any dose level. Similarly, reproductive organs weights (testes, epididymides and prostate/seminal vesicles) and sperm parameters (motility, morphology and counts) were unaffected by the treatment. Entecavir had no effect on reproductive function in male rats.

Embryo fetal development

Study title: 3. Entecavir: Oral study of embryo-fetal development in rats

Key study findings: presumed pregnant female rats received oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high). In this study, entecavir caused both maternal and embryo fetal toxicity at mid and high doses. Fetal malformation of the tail and vertebrae and developmental delays in ossification of the vertebrae, sternebrae and phalanges and increases in the number of lumbar vertebrae were seen at the high dose. In this study, a dose level of 2 mg/kg/day may be considered the NOAEL. Based on the body surface area factor, an equivalent oral dose in humans would be 0.32 mg/kg/day (19.5 mg/day for a 60 kg person).

Study no.: 910064395/97010

Volume #, and page #: 1 and 1-105

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, NJ

Date of study completion: August 18, 1997

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: RO22A and —

Methods

Doses: oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 2 (low), 20 (mid) or 200 mg/kg/day (high)

Species/strain: presumed pregnant rats (— . Sprague Dawley SD (BR);

Number/sex/group: 22 rats/group)

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: none

Study design: once daily dosing on gestation days 6 though 15 and then rats were necropsied on day 20 of gestation.

Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.

Results

Mortality (dams): one drug related death occurred (high) on day 16 of gestation. Necropsy revealed perioral and perinasal substance, edema of the stomach lining, fluid-filled stomach, thickened, dilated and discolored small intestine and enlarged adrenals. The dam had 14 early resorptions in utero. No other death occurred in the study (low or mid).

Clinical signs (dams): there were no drug related changes in animals (low or mid). Reduced and/or absent feces were noted in some dams (high).

Body weight (dams): drug related changes included lower maternal body weights and body weight gains (mid or high). No change in body weights, body weight gains or food consumption (low) was noted.

Food consumption (dams): decreased food consumption (high).

Postmortem observations: there were no drug related findings in dams (low) that survived to scheduled sacrifice on day 20 of gestation.

Maternal and litter observations at cesarean sectioning: there were no drug related findings in dams (low) that survived to scheduled sacrifice on day 20 of gestation. Drug related increases in embryo fetal death (resorptions) with associated decreases in live-litter sizes occurred in animals (mid or high). Lower fetal body weights were noted at the high dose.

Fetal observations: there were no drug related findings in dams (low) that survived to scheduled sacrifice on day 20 of gestation. High dose: drug related fetal malformations of the tail (constriction band, bent, short, stubbed or absent) and vertebrae (bifurcated arches) were observed. Developmental delays in ossification of the vertebrae (nonossified, incompletely ossified, irregular areas of ossification or unilateral ossification), sternebrae (nonossified) and phalanges (nonossified) were noted; all of which are expected observations in fetuses with mothers who had adverse effects on body weights. Drug related increases in minor skeletal variations (i.e., 7th lumbar vertebra and 14th rib) were observed.

Significantly increased incidence of several variations were observed exclusively in fetuses with severely reduced body weight (high litter # 4F0072; this litter had an average fetal weight of 1.44 g, as compared with an average of 3.02 g for the remaining dams in this

group; control group=3.88 g). These observations were considered by the sponsor secondary to the overall growth retardation by entecavir, rather than a direct effect of drug because they did not occur in any other fetuses and the litter incidence (considered a more valid parameter than fetal incidence) were not significantly increased as compared to the controls. Three of these fetuses had one or both kidneys which appeared small, while the remaining fetuses had incompletely or nonossified parietals, interparietal, tympanic annuli, ischia and pubes.

Study title: 4. Entecavir: Oral study of embryo-fetal development in rabbits

Key study findings: presumed pregnant female rabbits received repeated oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 1 (low), 4 (mid) or 16 mg/kg/day (high). In this study, statistically significant increases in the average number of ossified ribs (13^{th} rib) occurred at all doses tested.

Since statistically significant increases in the average number of ossified ribs (13th rib) occurred at all doses tested in this study, not withstanding the finding of single abnormality, there is a significant potential that entecavir could produce this abnormality in humans.

No drug related maternal changes were seen at any dose. Drug related increased in embryo-fetal death (resorptions) with associated decreases in liver-litter sizes occurred at the high dose. Approximately half of all conceptuses of does (high) were resorbed. Entecavir caused embryo-fetal toxicity (skeletal variant) at the low, mid or high dose without producing maternal toxicity.

Study no.: 910064861/97018

Volume #, and page #: 1 and 1-107

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick

Date of study completion: September 3, 1997

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Batch # RO22A and —

Methods

Doses: repeated oral gavage doses of entecavir at dose levels of 0 (avicel/methanol, vehicle control), 1 (low), 4 (mid) or 16 mg/kg/day (high)

Species/strain: presumed pregnant New Zealand White rabbits

Number/sex/group: 20 dams/group

Route, formulation, volume, and infusion rate: 5 ml/kg; oral gavage

Satellite groups used for toxicokinetics: none

Study design: once daily on gestation days 6 though 18. Rabbits were sacrificed on gestation day 29.

Parameters and endpoints evaluated: maternal gross necropsy and Cesarean-sectioning and fetal evaluations.

Results

Mortality (dams): one doe (low and mid) died following intubation accidents on day 12 or 15 of gestation. Both does were pregnant and had 6-10 embryos in utero, all of which appeared normal for their developmental age at the time of maternal death. Abortion of one or more conceptuses on days 19-23 of gestation occurred in one doe in each of the controls, mid and high dose groups. Although no drug related gross lesions were identified at necropsy of the high dose doe, all 10 conceptuses in the litter were resorptions. Necropsy findings of bright red lungs with dark red speckles were noted in the control doe and red perivaginal substance associated with abortion was seen in the mid dose doe. The litters of the control and mid dose does had 1 or 8 fetuses, all of which appeared normal for their developmental age. No other rabbit died or were sacrificed prior to scheduled termination.

Clinical signs (dams): there were no drug related changes in animals (low, mid or high).

Body weight (dams): there were no drug-related changes in maternal body weights, body weight gains or food consumption at any dose tested.

Postmortem observations: there were no drug related findings at necropsy of the does that survived to scheduled sacrifice on day 29 of gestation.

Maternal and litter observations at cesarean sectioning: there were no drug-related changes in cesarean-sectioned dams or their fetuses (low or mid). High dose: drug related increases in embryo fetal death (resorptions) with associated decreases in live-litter sizes occurred in the animals. Approximately half of all conceptuses (high) were absorbed. No other drug-related changes were observed at the high dose.

Offspring (malformations, variations, etc.): fetuses of the high dose group dams had developmental delays in ossification of the hyoid and an increased number of ossified ribs (13^{th} rib), a skeletal variation. Small, statistically significant increases in the average number of ossified ribs (13^{th} rib) also occurred at both the low and mid doses. No other fetal alteration was considered drug related. There were no other fetal findings of any kind at the low or mid doses. A summary of embryo-fetal development study in rabbits is

shown in Table 84.

The statistically significant changes in the ribs at low, mid and high doses were not considered drug related by the sponsor because this skeletal variant is known to have considerable variability within control population (historical control data using the New Zealand White Rabbit).

Table 84
Summary of observations in the embryo-fetal development study in rabbits (20 dams/group)

Toxicology parameters	Control (0 mg/kg/day)	Low (1 mg/kg/day)	Mid (4 mg/kg/day)	High (16 mg/kg/day
Clin Observations	None	none	none	None
Mortality	1	1	2	1
Gravid uterus weights (kg)	0.5	0.49	0.53	0.36*
Postimplantation loss (%)	4.3	2.8	5.4	49**
Litter size (live+dead)	7.8	7.9	8.7	4.8*
Resorptions (early+late)	3	4	7	84**
Litters with one or more live fetus on day 29	18	16	17	18
Mean live fetuses	7.7	7.9	8.7	4.8*
% Fetuses with any alterations	14.4	14.3	18.2	33.7**
% Fetuses with any variation	14.4	13.5	18.2	33.7
Hyoid: body Incomplete ossification: fetal incidence (%)	1.4	2.4	2	8.2*
Ossified ribs/fetus/litter (total)	12.61	12.81*	12.96**	13.01**

* = significantly different from control at $p < 0.05$
** = significantly different from control at $p < 0.01$

Study title: 5. Entecavir: one-week toxicokinetics study in pregnant rabbits

Key study findings: two groups of presumed pregnant rabbits received repeated oral gavage doses of entecavir at dose levels of 4 (mid) or 16 mg/kg/day (high) once daily on days 6 through 12 of gestation. On day 13 of gestation, the rabbits were sacrificed and necropsied, and pregnancy statuses were confirmed. Plasma concentrations were determined for all pregnant rabbits. Cmax was achieved at a median Tmax value of 0.5 hr. Mean Cmax and AUC24 hr values were 1.7 and 7.3 μg/ml and 5.6 and 23.4 μg*hr/ml at mid and high doses, respectively. Overall, the results indicated that there were dose related systemic exposures of pregnant rabbits to entecavir during the one week toxicokinetic study.

Study no.: 97029

Volume #, and page #: 1 and 102

Conducting laboratory and location: Bristol-Myers Squibb, New Brunswick, New Jersey

Date of study completion: November 6, 1997

GLP compliance: Yes

QA reports: yes

Drug, lot #, and % purity: RO22A and —

Methods

Doses: repeated oral gavage doses of entecavir at dose levels of 4 (mid) or 16 mg/kg/day (high).

Species/strain: presumed pregnant New Zealand White rabbits

Number/sex/group: 6 dams/group;

Route, formulation, volume, and infusion rate: 5 ml/kg; oral gavage

Satellite groups used for toxicokinetics: only toxicokinetics

Study design: once daily on gestation days 6 though 12. Plasma samples were obtained from each rabbit at 0.5, 1, 2, 4, 8 and 24 hr after dosing on gestation day 12.

Parameters and endpoints evaluated: toxicokinetics.

Results

Mortality (dams): Of the 6 rabbits assigned to the mid dose group, one was not pregnant, and one was removed from the study on day 10 of gestation because it was inadvertently not fed its daily ration of food and had a resulting body weight loss of 110 g from day 10 to 11 of gestation. Additionally, one of the six (high) was found dead on day 10 of gestation following an intubation accident. There were no remarkable observations in this study.

Toxicokinetics: the results are shown in Table 85. Cmax was achieved at a median Tmax value of 0.5 hr. Overall the results indicated that there were dose related systemic exposures of pregnant rabbits to entecavir during the one-week toxicokinetic study.

Table 85
Mean Cmax and AUC values in pregnant rabbits

Dose (mg/kg/day)	Cmax (μg/ml)	AUC 0-24 hr (μg*hr/ml)
4 (mid); n=4	1.7	5.6
16 (high); n=5	7.3	23.4

Prenatal and postnatal development

Study title: 6. Entecavir: Oral study of pre- and postnatal development in rats

Key study findings: Groups of presumed pregnant rats (25/group) received entecavir via oral gavage at dose levels of 0 (vehicle controls), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) from day 6 of gestation through day 20 of lactation. At the high dose level, a transient and mild reduction in maternal body weight gain occurred during gestation. There were no drug related findings in the F1 generation offspring at any dose level. Entecavir caused drug related changes in the F0 dams at the high dose level, without affecting the pre- or postnatal development of the F1 generation offspring at any dose level. A dose level of 3 mg/kg/day may be considered the NOEL for F0 dams. Based on the body surface area factor, an equivalent oral dose in humans would be 0.48 mg/kg/day (29.2 mg/day for a 60 kg person). A dose level of 30 mg/kg/day may be considered the NOEL for F1 offsprings. Based on the body surface area factor, an equivalent oral dose in humans would be 4.87 mg/kg/day (292 mg/day for a 60 kg person).

Study no.: 920009337/DN00005

Volume # and page #: 1 and 421

Conducting laboratory and location: [redacted]

Date of study completion: November 22, 2000

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: # RO22A, [redacted]

Methods

Doses: 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) once daily

Species/strain: presumed pregnant rats [redacted]: CD (SD) IGS BR VAF/Plus

Number/sex/group: 25 rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10ml/kg

Satellite groups used for toxicokinetics: No

Study design: presumed pregnant female rats received repeated oral gavage doses of entecavir at dose levels of 0 (sterile water, vehicle control), 0.3 (low), 3 (mid) or 30 mg/kg/day (high) once daily from day 6 of gestation through day 20 of lactation. Dams were allowed to deliver naturally and the litters were monitored for viability at birth, postnatal survival, appearance and growth. On day 21 of lactation, randomly selected F1 generation pups from each litter were continued on the study and the F0 generation dams and remaining F1 generation pups were euthanized. F1 generation rats that continued on study were evaluated for sexual maturation, sensory perception, motor activity learning, memory and reproductive function. At approximately 90 days of age, the F1 generation rats were evaluated for reproductive capacity. Within each dose group, rats were assigned to cohabitation, one male per female rat. The cohabitation period consisted of a maximum of 21 days. Female rats were Caesarean-section on gestation day 21 and the F2 generation fetuses were evaluated.

Parameters and endpoints evaluated:

Results

F_0 Generation rats

Mortality, clinical sign and necropsy observation: all rats survived to scheduled necropsy on day 21 of lactation. There were no necropsy findings.

Body weights and body weight changes: there were no drug related changes occurring in the F0 generation dams (low and mid). At the high dose, a transient and mild reduction in maternal body weight gain occurred during gestation.

Feed consumption: there were no drug related effects on absolute or relative maternal food consumption at any dose tested.

Natural delivery and litter observations: no effects on natural delivery or litter observations were observed at any dose. All pregnant rats delivered litters.

Clinical and necropsy observation-F_1 generation litter: There were no drug related findings in the F1 generation offsprings at any dose level.

F_1 generation rats:

Mortality: there were no drug related deaths. All F1 generation male and female rats survived to scheduled necropsy.

Clinical signs and necropsy observation in rats surviving to scheduled necropsy: no drug

related clinical observations occurred in either males or females at any dose level. No drug related gross lesions were identified at scheduled necropsy.

Testes and Epididymides weights: absolute and relative weights of the testes and Epididymides in the F1 generation male rats were unaffected.

Body weights, body weight changes and feed consumption: were unaffected by administration of entecavir to the F0 generation dams.

Postweaning behavioral evaluations: there were no drug related changes in the F1 generation rats when evaluated during the postweaning period for auditory startle, motor activity and watermaze learning and retention.

Sexual maturation: there were no changes in the age of preputial separation in F1 generation male rats or vaginal patency in F1 generation females.

Mating and fertility: were unaffected.

Caesarean sectioning and litter observations: entecavir had no effect on caesarean sectioning parameters in the F1 generation dams or the F2 generation litter. Values for corpora lutea, implantations, litter sizes, liver or dead fetuses, resorptions, fetal sex ratios and fetal body weighs were comparable for all groups.

F_2 findings: there were no drug related fetal gross external alterations in the F2 generation fetuses.

2.6.6.9 Discussion and Conclusions

Single dose studies

Single-dose oral toxicity studies were conducted in mice and rats at doses ranging from 40 to 5000 mg/kg. These studies were conducted in compliance with GLP regulations. Entecavir demonstrated a minimal order of acute toxicity in both species as 200 mg/kg was well tolerated. Doses of 1000 and 5000 mg/kg were overtly toxic and caused death in mice. The acute maximum tolerated single oral gavage dose of entecavir in the mouse was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg or 973 mg/day (60 kg person). Doses of 1000 and 5000 mg/kg were overtly toxic and caused death in rats. The acute maximum tolerated single oral gavage dose of entecavir in the rat was 200 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 32.46 mg/kg or 1.94 mg/day (60 kg person).

Repeat dose studies

Repeat-dose oral toxicity studies were conducted in mice and rats for up to 6 months, in dogs for up to 3 months, and in monkeys for up to 1 year; these species are routinely used in the safety evaluation of new chemical entities. All pivotal studies were conducted in

conformance with appropriate ICH guidelines and GLP regulations. Multiples of human exposures achieved in the multiple dose animal toxicology studies are shown in Table 86.

Table 86
Entecavir exposures in multiple dose toxicity studies in animals vs humans

Study and species	Dose (mg/kg/day)	AUC24hr (ng*h/ml)	NOEL/NOAEL (mg/kg/day)	Multiples of human exposures (x)	
Human (multiple dose)	0.5 mg	14.97	-	Based on AUC 0.5 mg	Based on AUC 1.0 mg
	1.0 mg	26.55	-	-	-
2 wk rats	2	745	2	50	28
	20	7166			
	200	70929			
2 wk dogs	1	4736	1	316	178
	10	80287			
	100/50	262664			
3 month dogs	0.3	1343	0.3	90	51
	3	12168			
	15	68020			
6 month rats	0.02	-			
	0.08	40			
	0.3	139	0.3	9	5
6 month mice	0.2	329	Not identified	22	12
	1	760			
	5	1229			
12 month monkeys	0.4	66			
	4	537			
	40	3620	40	242	136

The results of repeated dose toxicology studies indicated that the maximum therapeutic dose of 1.0 mg is supported by the NOELs/NOAELs achieved in the studies and entecavir exposure multiples at the NOELs/NOAELs are higher than that of clinical dose. The following principal target organs/tissues in animal studies were identified:

CNS

CNS inflammation was observed in a dose related manner in a 3 month dog toxicology study. The CNS changes appeared to be species specific as it was not observed in rats, mice or monkeys. Mean steady-state 24-hr exposures in mice, rats, and monkeys, at doses evaluated in repeat-dose studies in mice, dogs and monkeys exceeded the threshold exposure for CNS changes in dogs (Table 1). The exposure to entecavir at the no-effect dose for CNS changes was 13 times that in humans. In clinical trials, there were no reported events of drug-induced CNS toxicity, such as meningitis or encephalitis, or events suggestive of CNS injury such as seizures or cerebrovascular accidents. In addition, the frequency of CNS symptoms, including headache and dizziness, was similar in the entecavir vs comparator arms in Phase II/III studies.

Kidney

Kidney changes in mice (spontaneous nephropathy) and dogs (renal tubular degeneration) were observed only at overtly toxic doses in 6- and 3-month studies, respectively. No kidney lesions were observed in rats and monkeys at the highest doses tested in 6-month and 1-year studies, respectively. Nephrotoxicity was not reported in clinical trials with entecavir. This is an important finding given that the kidney is a major organ for the excretion of entecavir. The fact that kidney changes in animals occurred only at overtly toxic doses in 2 of the 4 species used in the safety assessment of entecavir and the lack of nephrotoxicity in clinical trials suggest that the kidney is not a target organ in humans.

Liver

In mice and rats, centrilobular degeneration in the liver (without associated increases in serum transaminases) was observed at ≥0.2 and 0.02 mg/kg, respectively. Liver degeneration was not evident in dogs (even at an overtly toxic dose) or monkeys at exposures to entecavir that were higher than those associated with liver changes in rodents; consequently, entecavir-induced liver degeneration appears to be a rodent-specific finding. In addition, given that the liver is the primary organ involved in hepatitis B infection, liver enzymes and measures of liver synthetic function were closely monitored in all clinical trials with entecavir. The frequency of on-treatment elevations in hepatic transaminases was similar across entecavir and comparator treatment groups.

Lung

In mice, alveolar histiocytosis was observed in the lungs at ≥1 mg/kg; the lung was also a target organ in the carcinogenicity study in mice at doses ≥0.04 mg/kg in males and at 4 mg/kg in females (discussed later). No similar changes were observed in repeat-dose studies in rats, dogs, or monkeys at exposures to entecavir that exceeded those in mice associated with lung changes (Table 86). The lung was not a target organ in clinical trials with entecavir.

Skeletal Muscle

Skeletal muscle myopathy occurred in mice at doses ≥1 mg/kg and in rats at doses ≥0.6 mg/kg in 6-month studies. Exposures to entecavir at no-effect doses for these findings were ≥4 times those in humans (Table 86). Skeletal muscle was not a target tissue in dogs or monkeys wherein exposures to entecavir exceeded those in rodents associated with skeletal muscle changes; thus, skeletal muscle myopathy appears to be specific to rodents.

Testes

In dogs, decreased weight of the testes was observed at 0.3 mg/kg and seminiferous-tubular degeneration was observed at ≥3 mg/kg (0.3 and 3 mg/kg were non-overtly toxic doses); these changes showed evidence of reversibility at 15 mg/kg following a 3-month

postdose period. The testes were not a target organ in monkeys at doses as high as 40 mg/kg. Thus, the only species that showed microscopic changes in the testes at non-overtly toxic doses was the dog; at the threshold dose for microscopic changes in the testes (3 mg/kg), systemic exposure to entecavir was 813 times higher than in humans at 0.5 mg and 458 times higher at 1 mg (Table 86).

Genetic toxicology

The genotoxic potential of entecavir was evaluated in a battery of well established and validated in vitro and in vivo test systems. The scope of the overall battery of tests and the individual study designs were in conformance with applicable ICH guidelines. Summary of results is shown in Table 87.

Table 87
Summary results of genotoxicology studies of entecavir

Type of study	Species strain	Method of administration	Duration of dosing	Doses (mg/kg)	Results
Genotoxicity in vitro					
1. Ames assay	S. typhimurium &E. coli	In vitro with/without S-9	46-48 hr	0,312.5, 625, 1250, 2500, 5000 μg/ml	Negative
2. Transformation of Syrian hamster embryo cells assay	Primary cells from Syrian hamster embryos	In vitro	7 days	0, 0.125, 0.25, 0.50, 1.0, 2 μg/ml	Negative
3. Cytogenetics study in primary human lymphocytes	Human chromosomes	In vitro with/without S-9	5-24 hr	2.5, 5, 10, 20, 50 or 200 μg/ml	Positive, clastogenic 10 μg/ml (without S-9) 50 μg/ml (with S-9)
Genotoxicity in vivo					
4 Oral DNA repair study in rats	Male rats	Po	2-16 hr	0, 2, 20, 200 or 2000 mg/kg	Negative
5. Oral micronucleus study in rats	Male rats	Po	3 days	0, 2, 20, 200 or 2000 mg/kg	negative

The notable finding is that in an in vitro chromosomal aberration test in primary human lymphocytes (without metabolic activation), entecavir was clastogenic at >10 μg/ml (36 μM) and the mitotic index was reduced >64% at these concentrations. Entecavir was negative in rest of the battery of tests.

Carcinogenicity studies

For detail see APPENDIX/ATTACHMENTS: #1 (review of the rat and mouse carcinogenicity studies) and # 2 (minutes of the Exec CAC) and #3 (minutes of the full CAC).

Carcinogenicity studies in CD-1 mice and SD rats were conducted in conformance with ICH Guidelines and in compliance with GLP regulations. CD-1 mice and SD rats are routinely used in carcinogenicity studies, and were the same strains utilized in pivotal repeat-dose toxicity studies with entecavir. The carcinogenicity studies were adequately designed, and included verification of exposure at all dosage levels. Dose selection for the carcinogenicity studies was based on ICH guidelines.

The following neoplasms were seen in rats and mice (Tables 88 and 89). In these studies, Entecavir was found to be a carcinogen in rodents.

Table 88

Incidence (%) of rat neoplasms in entecavir carcinogenicity studies. Multiple of exposure relative to a 1.0 mg dose in humans is shown in parentheses

Neoplasm	Sex	0	Low	Mid	high	Highest	Historica control ran
Brain glioma	Male	0	2%(0)	2%(0.3)	3%(5)	7%*(35)	0-3%
Brain glioma	Female	0	0(0.4)	2%(1)	0(4)	5%*(24)	0-1.5%
Liver adenoma	Female	1%	4%	5%	2%	13%**	0-5%
Liver carcinoma	Female	0	0	0	0	5%	0%
COMBINED	Female	1%	4%	5%	2%	18%**	
Skin fibroma	Female	0	0	2%	3%	5%*	0-7%

* = statistically significant for rare tumors at $p < 0.025$
** = statistically-significant tumors determined using Peto analysis for common tumors at $p < 0.005$
d = despite high incidence, not significant using Peto analysis for common tumor at $p < 0.005$
na = not available

Table 2

Incidence (%) of certain neoplasms in mice entecavir carcinogenicity studies. Multiple of exposure relative to a 1.0 mg dose in humans is shown in parentheses

Neoplasm	Sex	0	Low	mid	high	Highest	Historica control ran
Lung adenomas	Male	7%	13%(1)	19%**(3)	28%**(13)	33%**(42)	6-31%
Lung carcinoma	Male	5%	7%	7%	12%	25%**	0-14%
Combined	Male	12%	20%	26%**	40%**	58%**	
Liver adenoma	Male	10%	7%	3%	13%	12%	na
Liver carcinoma	Male	1%	2%	5%	3%	13%**	0-16%
COMBINED	Male	11%	9%	8%	16%	25%**	
Lung adenomas	Female	13%	8%(1)	7%(3)	27%d(10)	25%**(40)	3-16%
Lung carcinoma	Female	7%	5%	3%	8%	27%**	2-14%
Combined	Female	20%	13%	10%	35%	52%**	
Liver adenomas	Female	0	3%	0	2%	3%	na
Liver carcinomas	Female	0	0	2%	0	0%	Na
COMBINED	Female	0	3%	2%	2%	3%	
Ovaries: Hemangioma:	Female	5%	10%	12%	8%	32%**	0-7%
Uterus: Hemangioma:		6%	7%	7%	8%	13%**	0-6%
Spleen: Hemangiosarcoma		1%	0	3%	3%	3%** 16%**	1-2%
Hemangioma/hemangiosarcoma		4%	6%	7%	7%		0-7%

* = statistically significant for rare tumors at $p < 0.025$

** = statistically-significant tumors determined using Peto analysis for common tumors at $p < 0.005$

d = despite high incidence, not significant using Peto analysis for common tumor at $p < 0.005$

na = not available

Reproductive and developmental toxicology

A complete battery of reproductive toxicity studies was conducted with entecavir. All pivotal studies were conducted in compliance with GLP regulations, were adequately designed, and met ICH guidelines. Systemic exposures to entecavir in support of the reproductive studies were established using either existing toxicokinetic data from toxicology studies (rats) or the results of a separate toxicokinetics study in pregnant rabbits. Oral studies of fertility and early embryonic development were conducted separately in males and females because different doses were used in these. Multiples of

human exposures achieved in the reproductive toxicology studies are shown in Table 89.

Table 89
Entecavir exposures in reproductive toxicity studies in rats and rabbits vs humans

Study and species	Dose (mg/kg/day)	AUC24hr (ng*h/ml)	NOEL/NOAEL (mg/kg/day)	Multiples of human exposures (x)	
Human (multiple dose)	0.5 mg	14.97	-	Based on AUC 0.5 mg	Based on AUC 1.0 mg
	1.0 mg	26.55	-	-	-
Rat male Seg I	0.1				
	1				
	10	>2396	10	>160	>90
Rat female Seg I	0.3				
	3				
	30	>2483	30	>165	>94
Rat female Seg II	2	745	2	50	28
	20				
	200				
Rat Seg III	0.3				
	3				
	30	>2483	30	>165	>94
Rabbit Seg II	1		ossification sites/fetus/litter: ribs (12.81, p=0.05)	Not measured	Not measured
	4	5639	12.96 , p=0.01	377	212
	16	23448	13.01, p=0.01	1566	883

In reproductive and developmental toxicity studies in rats, entecavir demonstrated no effects on reproductive function or fertility, and no adverse findings in a perinatal/postnatal study at exposures ~28 times that in humans at 1 mg daily. In the pivotal embryo-fetal development study in rats wherein doses of 2, 20, and 200 mg/kg were evaluated, both maternal and embryo-fetal toxicities were evident at ≥20 mg/kg (eg, decreased maternal body weights at ≥20 mg/kg, 1 maternal death at 200 mg/kg, increased resorptions at ≥20 mg/kg, and malformations of the tail and vertebrae at 200 mg/kg). The exposures to entecavir in female rats at the no-effect and threshold doses for toxicity are 28 and 180 times that in humans at 1 mg daily, respectively. In the pivotal embryo-fetal development study in rabbits wherein doses of 1, 4, and 16 mg/kg were evaluated, no evidence of maternal toxicity was evident at any dose. There was an increased incidence of resorptions with associated decreases in live-litter sizes, developmental delays in ossification of the hyoid, and an increased incidence of 13th rib in dose related manner. Thus, entecavir was a selective developmental toxicant in rabbits. A NOAEL could not be identified for embryo-fetal development in rabbits.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: To support clinical use, the nonclinical toxicity profile of entecavir was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in rats and mice. The pivotal toxicology studies supporting the safety of entecavir were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of entecavir in humans.

Unresolved toxicology issues (if any): None

Recommendations: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

Suggested labeling: The issue of labeling will be carried out separately.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS:

#1 Exec CAC minutes pertaining to the rat and mouse carcinogenicity studies
#2 Full CAC minutes pertaining to the rat and mouse carcinogenicity studies

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Pritam Verma
3/25/05 08:24:31 AM
PHARMACOLOGIST

James Farrelly
3/29/05 08:57:10 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-798**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09/30/04**
PRODUCT:	**Entecavir**
INTENDED CLINICAL POPULATION:	**Chronic Hepatitis B Virus infection**
SPONSOR:	**Bristol-Myers Squibb Company**
DOCUMENTS REVIEWED:	**Electronically**
REVIEW DIVISION:	**Division of Antiviral Drug Products (HFD-530)**
PHARM/TOX REVIEWER:	**Pritam S. Verma, Ph.D.**
PHARM/TOX SUPERVISOR:	**James G. Farrelly, Ph.D.**
DIVISION DIRECTOR:	**Debra Birnkrant, M.D.**
PROJECT MANAGER:	**Marsha Holloman**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

B. Recommendation for nonclinical studies: To support clinical use, the nonclinical toxicity profile of entecavir was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in rats and mice. The pivotal toxicology studies supporting the safety of entecavir were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of entecavir in humans.

C. Recommendations on labeling: The issue of labeling will be carried out separately.

II. Summary of nonclinical findings

A: Brief overview of nonclinical findings: Entecavir is an antiviral agent that is being developed for the treatment of chronic hepatitis B virus infection in humans. The recommended doses of entecavir are 0.5 mg (AUC = 14.97 ng*hr/ml) daily in nucleoside-naive patients and 1 mg (AUC = 26.55 ng*hr/ml) daily in lamivudine refractory patients.

The pharmacokinetic (PK) characteristics of entecavir in mice, rats, rabbits, dogs, and monkeys are comparable to those in humans indicating the acceptability of these species for the toxicological assessment of entecavir. The effective T-half in humans is approximately 24 hr. Serum protein binding of entecavir was low across animal species and humans (ranging from 8 to 24%), suggesting that there is little potential for drug interactions by displacement of other highly protein-bound drugs from their protein binding sites.

In both animals and humans, entecavir is almost entirely eliminated through the kidneys with parent drug as the major form present in urine. Only Phase II metabolites (glucuronides and sulfates) of entecavir were found in animals and humans and all of the metabolites identified in humans were present in the species used in the toxicological evaluation of entecavir. The total amount of metabolites, as a percentage of the total radioactive dose in excreta (urine and feces), was low in all species studied (eg, ~30% in animals and ~10% in humans). Entecavir is not a substrate, inhibitor, nor an inducer of the CYP isozymes and the PK of entecavir will neither effect nor be affected by the co-administration of agents that are either metabolized by, inhibit, or induce the CYP system.

Species-specific, reversible CNS inflammation was seen in dogs administered doses that achieve ~51 times the exposure to entecavir in humans at clinically proposed doses. The

species specificity, reversibility, and high exposure multiples at which the CNS inflammation was seen lead to the conclusion that this is probably not relevant to human safety. Other target organs in repeat-dose studies in animals were the kidneys, liver, lungs, skeletal muscle and testis; the changes in these organs were considered unlikely to be relevant to human safety because they were either species specific, associated with high exposure multiples relative to humans, and/or, in clinical trials with entecavir, they were not target tissues. The data from the 1-year study in monkeys indicated that there was no target organ toxicity in monkeys at exposures to entecavir ~136 times those in humans.

In a battery of genetic toxicology studies, entecavir was negative, with the exception of an in vitro chromosomal aberration test in primary human lymphocytes (without metabolic activation). These data indicate that entecavir is a genotoxic molecule. Carcinogenicity studies in CD-1 mice and SD rats were conducted. Increased incidence of tumors was observed in both the studies. The results of these studies were presented to the Executive Carcinogenicity Assessment Committee (ECAC) on June 17, 2003. The outcomes of the two studies were as follows:

Rat Carcinogenicity Study: The oncogenicity potential of entecavir was investigated in male rats at oral gavage dosages of 0.003 (low), 0.02 (mid), 0.2 (high) or 1.4 mg/kg/day (highest) and in females at dose levels of 0.01 (low), 0.06 (mid), 0.4 (high) or 2.6 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The Executive CAC committee found that the study was adequately designed and conducted.

The NOEL for neoplasia was 0.2 mg/kg/day for males and 0.06 mg/kg/day for females.

At tumorigenic doses, systemic exposures were 35- and 4-times that in humans (1.0 mg daily dose) in male and female rats, respectively.

Treatment-Associated Tumors:

1. Hepatocellular adenomas in female rats were significant ($p=0.005$) at the highest dose level. Combined adenomas and carcinomas in the female rats were also significant ($p=0.005$) at the highest dose. In female rats, the combined incidence of adenomas and carcinomas was 1% (controls), 4% (low), 5% (mid), 2% (high) and 18% (highest).

2. Brain gliomas were significant ($p=0.025$) at the highest dose in both male and female rats. In male rats, the incidence was 0% (controls), 2% (low), 2% (mid), 3% (high) and 7% (highest). In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 0% (high) and 5% (highest).

3. The skin fibromas in female rats were significant ($p=0.025$) at the high and highest doses. In female rats, the incidence was 0% (controls), 0% (low), 2% (mid), 3% (high) and 5% (highest).

Mouse Carcinogenicity Study: The oncogenicity potential of entecavir was investigated in mice at oral gavage dosages of 0.004 (low), 0.04 (mid), 0.4 (high) or 4.0 mg/kg/day (highest) in comparison with vehicle controls for a period of 104 weeks. The Executive CAC committee found that the study was adequately designed and conducted.

The NOEL for neoplasia was 0.004 mg/kg/day for males, based on pulmonary adenomas; for all other tumors in males and females, the NOEL was 0.4 mg/kg/day. At the tumorigenic dose in male mice, systemic exposure was 3-times that in humans (1.0 mg daily dose).

Treatment-Associated Tumors:

1. Lung adenomas were significant (p=0.005) in male mice (mid, high and highest) and in the female mice at the highest dose (p=0.005); lung carcinomas in both male and female mice were significant (p=0.005) at the highest dose. Combined lung adenomas and carcinomas were significant (p=0.005) in male mice at the mid, high and highest dose levels and in the female at the highest dose level (p=0.005). In male mice, the combined incidence of adenomas and carcinomas was 12% (controls), 20% (low), 26% (mid), 40% (high) and 58% (highest). In female mice, the combined incidence of adenomas and carcinomas was 20% (controls), 13% (low), 10% (mid), 35% (high) and 52% (highest).

2. Hepatocellular carcinomas in male mice were significant (p=0.005) at the highest dose level. In male mice, the combined incidence of adenomas and carcinomas was 11% (controls), 9% (low), 8% (mid), 16% (high) and 25% (highest).

3. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant (p=0.005) at the highest dose level. In female mice, the incidence of vascular tumors was 16% (controls), 23% (low), 29% (mid), 26% (high) and 64% (highest).

The ECAC found that the carcinogenicity studies in mice and rats were adequately designed and conducted. The committee judged the results of entecavir carcinogenicity studies. They concluded that entecavir was a carcinogen in rodents. The committee concluded that entecavir produced tumors in both species and both genders, and these results suggest a potential cancer hazard to patients.

At the request of the sponsor, the results of the carcinogenicity studies were presented to the full CAC (CAC), the committee that has been designated as the arbiter of disputes between sponsor and review divisions regarding the relevance of results in carcinogenicity studies. The CAC met on January 7, 2005 and voted that hepatocellular adenomas and carcinomas in female rats, skin fibromas in female rats and brain gliomas in both male and female rats were relevant. The committee also agreed that in the mouse carcinogenicity study, liver tumors in males and vascular tumors in females as well as lung tumors in both sexes were relevant to human safety evaluation.

In reproductive and developmental toxicity studies in rats, entecavir demonstrated no

effects on reproductive function or fertility, and no adverse findings in a perinatal/postnatal study at exposures > 90 times that in humans at 1 mg daily. In the pivotal embryo-fetal development study in rats wherein doses of 2, 20, and 200 mg/kg were evaluated, both maternal and embryo-fetal toxicities were evident at >20 mg/kg (eg, decreased maternal body weights at >20 mg/kg, 1 maternal death at 200 mg/kg, increased resorptions at > 20 mg/kg, and malformations of the tail and vertebrae at 200 mg/kg). The exposures to entecavir in female rats at the no-effect and threshold doses for toxicity are 28 and 212 times that in humans at 1 mg daily, respectively. In the pivotal embryo-fetal development study in rabbits wherein doses of 1, 4, and 16 mg/kg were evaluated, no evidence of maternal toxicity was evident at any dose. There was an increased incidence of resorptions with associated decreases in live-litter sizes, developmental delays in ossification of the hyoid, and an increased incidence of 13th rib in dose related manner. Thus, entecavir was a selective developmental toxicant in rabbits. A NOAEL could not be identified for embryo-fetal development in rabbits.

B. Pharmacological activity: Entecavir is efficiently phosphorylated to entecavir-triphosphate (TP) by cellular nucleoside kinases. By competing directly with the natural deoxyguanosine triphosphate (dGTP), entecavir-TP potently inhibits each of the 3 distinct activities of the HBV viral polymerase: priming, reverse transcription of first-strand DNA synthesis, and the DNA-dependent DNA polymerase activity responsible for second-strand DNA synthesis.

C. Nonclinical issues relevant to clinical use: The Exec CAC concluded that entecavir produced tumors in rats and mice and both genders, and these results suggest a potential cancer hazard to patients. Entecavir can be classified as Pregnancy Category C. Entecavir should be used during pregnancy only if clearly needed.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

Entecavir (BMS-200475), a cyclopentyl guanosine analog, is an inhibitor of hepatitis B virus (HBV). The triphosphate form of entecavir blocks human HBV reverse transcriptase activity, inhibiting both the priming and elongation steps of the virus replication. Entecavir is being developed for the treatment and prevention of chronic HBV infection in adults.

The oral bioavailability of entecavir is approximately 37% to 80% in rats, 91% or greater in dogs and 16% in monkeys. Following intravenous administration to rats and dogs, entecavir has a steady state volume of distribution (1.02 to 4.63 L/kg) that is higher than the total body water, which suggests extravascular distribution and tissue protein biding. The human serum protein binding of entecavir is low (approximately 13%) indicating that there is a very low potential for drug-drug interaction due to displacement of protein bound drugs when coadministered with entecavir. Studies in pregnant and lactating rats indicate that entecavir penetrated the placenta and distributed into the fetus and that entecavir and/or its metabolite(s) were secreted into milk. The studies also indicated that entecavir crossed the blood/brain barrier in pregnant female rats and the blood/testis barrier in male rats. One glucuronide and 2 sulfate conjugates of entecavir have been identified in the urine of rats, dogs and monkeys in vivo but the contribution of these metabolites to the overall metabolite profile is relatively small, especially in dogs and monkeys. entecavir is not an inhibitor of human cytochrome P450 isozymes 1A2, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4, suggesting that it does not have the potential to alter the human metabolic clearance of drugs that are metabolized by these major cytochrome P450 isozymes. The terminal elimination half-life after intravenous administration is 2.1 to 4 hr in rats, 3.8 to 9 hr in dogs and 6 hr in monkeys. A majority of the administered intravenous dose is recovered unchanged in the urine of rats (51% to 68%), dogs (72% to 76%) and monkeys (60%); after oral administration, the urinary recovery of the intact drug is 44% in rats, 63% to 83% in dogs and 12% in monkeys. This indicates that, in general, entecavir is cleared predominantly by the renal route in these animals.

Entecavir has been evaluated in a number of toxicology studies including single dose oral studies in rodents, 2-week, 3-month and 6-month oral studies in rodents, 3-month studies in dogs, and 3-and 12-month studies in monkeys. Entecavir was also tested for oral teratology studies in rats and rabbits, oral studies of fertility and early embryonic development and pre- and postnatal development in rats and battery of genetic toxicology studies. In addition, 2-year oral carcinogenicity studies and investigative studies with entecavir in mice and rats were performed. General toxicology studies in a variety of animals have shown that target organs for toxicity may include liver, bone marrow, lungs, kidneys, intestine, testes, and skeletal muscle. In a 6-month oral toxicology study of entecavir in mice at doses of 0.2, 1.0 or 5.0 mg/kg/day, liver was the target organ at all the doses. In addition, skeletal muscle and lung were the target organs at the mid or high doses. In a 6-month oral toxicology study of entecavir in rats at doses of 0.02, 0.08 or 0.3

mg/kg/day, degenerative changes in the liver were observed at all doses. A one year oral toxicity study in cynomolgus monkeys, revealed no significant toxicity other than minimal elevation of BUN and potassium at exposures of 125 to 1000-fold greater than those predicted of human studies. In dogs at dose levels of 0.3, 3.0 or 30 mg/kg/day, adverse effects were evident at all doses and organs/tissues known to be targets included bone marrow, testes, kidneys, prostate glands, liver and inflammation in the CNS was observed at all doses. CNS lesions were not seen in dogs that were sacrificed in moribund condition after 1-month of dosing. CNS lesions were also not seen in rodents or monkeys. The CNS lesion was shown to be reversible and appeared to be specific to dogs of all the species studied.

Two-year oral carcinogenicity studies in rats showed significant incidence of hepatocellular adenomas and carcinomas in females, brain gliomas in males and females, and skin fibromas in females at the highest dose. In the two-year oral carcinogenicity studies in mice, combined incidence of lung adenomas and carcinomas was significant in males and females. Combined incidence of hepatocellular adenomas and carcinomas in males were significant at the highest dose level. Vascular tumors in female mice (hemangiomas of ovaries and uterus and hemangiomas/ hemangiosarcomas of spleen) were significant at the highest dose level.

In an oral study of embryo-fetal development in rabbits, entecavir at dose levels of 1.0, 4.0 or 16.0 mg/kg caused embryo-fetal toxicity, delays in ossification of the hyoid and an increased incidence of 13th rib in a dose related manner. The exposures (AUCs) were 5.6 and 23.4μg*hr/ml at the mid and high dose, respectively. The exposures of rabbits to entecavir were 210 and 879 times, respectively, that in humans at the maximum therapeutic dose (1.0 mg, 26.6 ng*hr/ml). With regard to genotoxicity, entecavir was tested for its potential to induce chromosome aberrations in cultured human lymphocytes where it was positive and it was found to be clastogenic. Presently, the sponsor has submitted this application for review by the Division.

NDA number: 21-798

Review number: 000

Sequence number/date/type of submission: 000/0-30/N

Information to sponsor: No

Sponsor and/or agent: Bristol-Myers Squibb Company
5 Research Parkway
Wallingford, CT 06492

Manufacturer for drug substance: Bristol-Myers Squibb Company
One Squibb Drive
New Brunswick, NJ 08903-0191

Reviewer name: Pritam S. Verma, Ph.D.

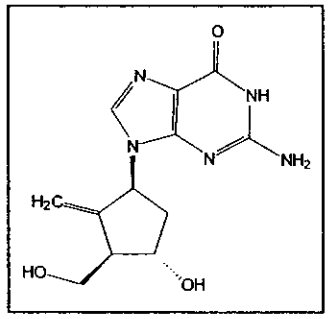
Division name: Division of Antiviral Drug Products

HFD #: 530

Review completion date: 1/26/05

Drug:

Trade name: BARACLUDE
Generic name: Entecavir
Code name: BMS-200475
Chemical name: 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one monohydrate
CAS registry number: 142217-69-4
Molecular formula: $C_{12}H_{15}N_5O_3.H_2O$
Molecular weight: 295.3
Structure:



Relevant IND: 52,196

Drug class: Nucleoside analog

Intended clinical population: Hepatitis B Virus (HBV) infected adults

Clinical formulation: oral solution, 0.05 mg/ml

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

NONCLINICAL TOXICOLOGY

All pharm/tox information contained in NDA 21-797 is incorporated by cross-reference to NDA 21-798 with the exception of the proposed label. Please see the review of NDA 21-797.

OVERALL conclusions and recommendations

Conclusions: To support clinical use, the nonclinical toxicity profile of entecavir was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in rats and mice. The pivotal toxicology studies supporting the safety of entecavir were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. The results of nonclinical toxicology studies

(other than carcinogenicity studies) do not suggest any special safety issues for humans taking entecavir. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of entecavir in humans.

Unresolved toxicology issues (if any): None

Recommendations: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

Suggested labeling: The issue of labeling will be carried out separately.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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/s/

Pritam Verma
3/25/05 08:40:03 AM
PHARMACOLOGIST

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