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Preclinical and Toxicology Studies of 1263W94, a Potent and Selective Inhibitor of Human Cytomegalovirus Replication

George W. Koszalka,[1] Nelson W. Johnson,[1] Steven S. Good,[1] Leslie Boyd,[1] Stanley C. Chamberlain,[1] Leroy B. Townsend,[2] John C. Drach,[2] and Karen K. Biron[1]*

GlaxoSmithKline, Research Triangle Park, North Carolina 27709,[1] and College of Pharmacy and School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109[2]

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1263W94 is a novel benzimidazole compound being developed for treatment of human cytomegalovirus infection. No adverse pharmacological effects were demonstrated in safety pharmacology studies with 1263W94. The minimal-effect dose in a 1-month rat study was 100 mg/kg/day, and the no-effect dose in a 1-month monkey study was 180 mg/kg/day. Toxic effects were limited to increases in liver weights, neutrophils, and monocytes at higher doses in female rats. 1263W94 was not genotoxic in the Ames or micronucleus assays. In the mouse lymphoma assay, 1263W94 was mutagenic in the absence of the rat liver S-9 metabolic activation system, with equivocal results in the presence of the S-9 mix. Mean oral bioavailability of 1263W94 was >90% in rats and ~50% in monkeys. Clearance in rats and monkeys was primarily by biliary secretion, with evidence of enterohepatic recirculation. In 1-month studies in rats and monkeys, mean peak concentrations and exposures to 1263W94 increased in near proportion to dose. Metabolism of 1263W94 to its primary metabolite, an N-dealkylated analog, appeared to be mediated via the isozyme CYP3A4 in humans. 1263W94 was primarily distributed in the gastrointestinal tract of rats but did not cross the blood-brain barrier. In monkeys, 1263W94 levels in the brain, cerebrospinal fluid, and vitreous humor ranged from 4 to 20%, 1 to 2%, and <1%, of corresponding concentrations in plasma, respectively. The high level of binding by 1263W94 to human plasma proteins (primarily albumin) was readily reversible, with less protein binding seen in the monkey, rat, and mouse. Results of these studies demonstrate a favorable safety profile for 1263W94.

Human cytomegalovirus (HCMV) is a herpesvirus that causes widespread infection. The virus generally persists in the host and can be reactivated in cases of immune suppression. HCMV infection is associated with significant morbidity and mortality in certain populations, including neonates and immunocompromised patients such as transplant recipients and AIDS patients (10). In the United States, drugs currently approved for treatment of HCMV infection include ganciclovir (GCV; Cytovene), cidofovir (CDV; Vistide), and foscarnet (PFA; Foscavir). For prophylaxis in solid organ transplant recipients or for prophylactic or maintenence treatment of HCMV retinitis in AIDS patients, GCV can be administered orally or by intravenous (i.v.) injection. For induction treatment of HCMV retinitis, GCV must be administered by i.v. injection; CDV and PFA can be administered only by i.v. injection. Two drugs available for the intravitreal treatment of HCMV retinitis in AIDS patients are Vitrasert, the intravitreal implant formulation of GCV, and Fomivirsen, an antisense-designed oligonucleotide administered via intravitreal injection. Finally, valganciclovir (Valcyte), the oral prodrug of ganciclovir, was recently approved for the treatment of HCMV retinitis in patients with AIDS.

Problems associated with each of these therapies include toxicities, such as bone marrow suppression (granulocytopenia, anemia, and thrombocytopenia), with GCV (8, 18) and nephrotoxicity with CDV (14, 15) and PFA (5). In addition, emergence of drug-resistant virus in patients undergoing long-term maintenance therapy with these drugs has resulted in diminished drug efficacies (12, 13). Because the approved drugs have similar mechanisms of action, the emergence of HCMV mutations associated with resistance to one drug often leads to resistance to the other drugs (4).

1263W94 (5,6,-dichloro-2-isopropylamino-1-β-L-ribofuranosyl-1H-benzimidazole; Fig. 1) is a member of a new class of drugs, the benzimidazole ribosides and is being developed for the treatment of HCMV. 1263W94 is a potent antiviral agent in vitro with an approximately 4- to 7-fold-lower mean 50% inhibitory concentration (IC_{50}) than GCV in the laboratory strain AD169 (0.12 versus 0.53 μM) and in 10 clinical HCMV isolates (0.03 to 0.13 μM versus 0.15 to 1.10 μM) as measured by DNA hybridization (3). Although one mechanism by which 1263W94 inhibits HCMV replication is by interfering with HCMV DNA synthesis, the inhibition differs from that of GCV, CDV, or PFA in that it does not involve HCMV DNA polymerase. In addition, 1263W94 is active against viral mutants that are resistant to GCV, CDV, and PFA (6). Since 1263W94 is being developed as a novel anti-HCMV agent, we report the results of several preclinical studies that were performed to assess the safety and pharmacokinetics of 1263W94.

MATERIALS AND METHODS

Compound. 1263W94 was synthesized at Wellcome Research Laboratories, Research Triangle Park, N.C., or Beckenham, Kent, United Kingdom. The purity of the compound used in these studies was >96%. [^{14}C]-labeled 1263W94 ([^{14}C]1263W94), used in the metabolism and excretion/mass balance studies and protein-binding studies, was synthesized at Wellcome Research Laboratories and had a radiochemical purity of >98%. [^{3}H]-labeled 1263W94 ([^{3}H]1263W94),

* Corresponding author. Mailing address: GlaxoSmithKline, 5 Moore Dr., Research Triangle Park, NC 27709. Phone: (919) 483-9310. Fax: (919) 315-5243. E-mail: kkb21173@gsk.com.

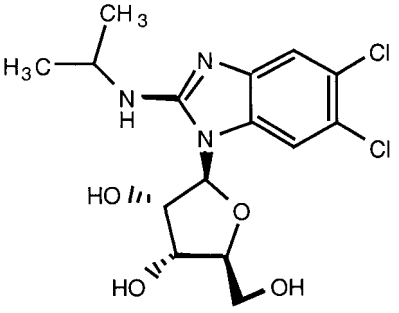

FIG. 1. Chemical structure of 1263W94 (molecular weight, 376.24).

used in the human in vitro metabolism study, was synthesized by Moravek Biochemicals, Inc., Brea, Calif.

Animals. Male and female CD-1 mice were obtained from Charles River Laboratories (Wilmington, Mass.). Male and female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.). Han Wistar rats were obtained from Glaxo Wellcome Research and Development (Bury Green, United Kingdom). Beagle dogs were obtained from Marshall Farms USA, Inc. (North Rose, N.Y.). Young adult male and female cynomolgus monkeys (*Macaca fascicularis*) were obtained from Charles River Primates (Houston, Tex.); HRP, Inc. (Alice, Tex.); or White Sands Research Center (Alamogordo, N.Mex.). All animal studies were conducted according to good laboratory practices or accepted scientific standards.

Drug administration. For safety pharmacology studies, 1263W94 was administered as a suspension in 0.5% methylcellulose (10 ml/kg) to mice via oral gavage and in citrate buffer (50 ml/kg) as an i.v. bolus dose via the right femoral vein to dogs. For acute toxicology studies, 1263W94 was prepared as a solution in citrate buffer (oral studies [citric acid, 1 N HCl, distilled water]; i.v. studies [citrate monohydrate, HCl or NaOH, sterile saline]). Oral doses (50 mg/ml) were administered by gavage, and i.v. doses (5 or 20 mg/ml) were administered via the lateral tail vein (mice and rats), or the right jugular vein (rats). For subchronic toxicology studies, 1263W94 was suspended in 0.5% methylcellulose for the 28-day dose range finding studies in rats (10 ml/kg) and monkeys (20 ml/kg). However, due to the lack of homogeneity and variable blood concentrations, the vehicle was changed to citrate buffer (citrate monohydrate, with 0.1 M NaOH for controls and 0.1 N HCl for drug-treated samples) for the definitive 1-month studies. For mutagenicity studies, 1263W94 was prepared as a solution in methanol-water (Ames assay), dimethyl sulfoxide (mouse lymphoma assay), and citric acid–sterile water–1 N HCl (micronucleus assay). For pharmacokinetic studies in rats and monkeys, 1263W94 was administered orally or i.v. as a solution in normal saline containing either (i) 10% (vol/vol) ethanol and 40% (vol/vol) polyethylene glycol 400 or (ii) 20 to 50% (vol/vol) propane-1,2-diol.

Safety pharmacology experiments. Safety pharmacology experiments were performed to assess the potential for 1263W94 to produce any adverse pharmacologic effects (Table 1). 1263W94 was tested at various single doses (10 to 300 mg/kg) or concentrations (3 to 30 μg/ml) in a broad array of in vitro and in vivo (mice and rats) pharmacological assays (PharmaScreen assays; Panlabs, Inc., Bothell, Wash.). In a separate in vitro experiment, the effects of 10 μM 1263W94 (3.8 μg/ml) on the contraction of guinea pig ileum ($n = 6$) by the cholinergic agonist, acetylcholine, or the histaminergic agonist, histamine, and of rabbit aortic tissues ($n = 6$) by the α_1-adrenergic agonist, *l*-norepinephrine, were compared with the same response in the presence of vehicle (ethanol). In an in vivo experiment, male CD-1 mice (four animals/group; average weight, 23 g) were fasted overnight and then given single oral doses of 250, 500, or 1,000 mg of 1263W94/kg. Animals were observed continuously for 4 h after dosing for overt effects and daily for 7 days (except weekends) to monitor the delayed effects of treatment. In another study, two groups of anesthetized beagle dogs (three animals/group; weight range, 10 to 13 kg) received vehicle or 3, 10, and 30 mg of 1263W94/kg as i.v. bolus injections in a cumulative fashion, allowing 30 min between doses, and then monitored continuously for cardiovascular and respiratory effects.

Toxicology experiments. Preclinical toxicity studies conducted with 1263W94 consisted of acute oral and i.v. studies in rats and mice, with 28-day dose range finding studies in rats and monkeys to identify doses for the definitive 1-month studies in these two species, and three genetic toxicology studies.

Acute toxicity studies. The single doses of 1263W94 given orally or i.v. to groups of male and female CD-1 mice (19 to 31 g) and Sprague-Dawley rats (126 to 243 g) are listed in Table 2. Animals were observed for viability and clinical signs on the day of dosing and daily thereafter for 14 days. Body weights were recorded on the day of dosing and at weekly intervals thereafter, and macroscopic examinations were performed on day 14 or at the time of death.

Subchronic toxicity studies. Preliminary 28-day oral studies with 1263W94 in Sprague-Dawley rats (four animals/sex/group) and cynomolgus monkeys (two animals/sex/group) established doses for the 1-month studies. In the 1-month studies, 1263W94 was administered orally to male and female Sprague-Dawley rats (118 to 171 g) at doses of 0, 100, 200, and 400 mg/kg/day and to cynomolgus monkeys (2.2 to 5.6 kg) at 0, 20, 60, and 180 mg/kg/day. These studies included groups of animals to determine reversibility, toxicokinetics, and histopathology (Table 3).

Genetic toxicity studies. Reverse mutation assays with *Salmonella enterica* serovar Typhimurium strains TA98, TA100, TA102, TA1535, and TA1537 were performed with or without metabolic activation (S9) to test 1263W94 at concentrations up to 650 μg/plate in the standard Ames assay (1) and at up to 205 μg/plate in the preincubation modification assay (16). 1263W94 was prepared as a solution in methanol-water (1:1), and appropriate positive controls were used. The mutagenic potential of 1263W94 was also tested in the in vitro mouse lymphoma mammalian cell mutagenesis assay (7). Four tests were performed, two with and two without S9, at maximum 1263W94 concentrations of 150 and 75 μg/ml, respectively. Dimethyl sulfoxide was the solvent, and methylcholanthrene and methyl methanesulfonate were the positive controls with and without S9, respectively. Finally, an in vivo micronucleus assay (11) was performed in Han Wistar rats (five animals/sex/group) given single oral doses of 1263W94 in 0.04 M citrate buffer (pH 2.5) at 0, 400, 800, and 1,200 mg/kg. The positive control, cyclophosphamide, was given as a single oral dose of 40 mg/kg. The numbers of micronuclei in bone marrow preparations obtained at 24 and 48 h postdose were determined.

Absorption, distribution, metabolism, and excretion. A series of oral and i.v. studies were performed with 1263W94 in Sprague-Dawley rats and cynomolgus monkeys. Pharmacokinetics and oral bioavailability were determined by admin-

TABLE 1. Safety pharmacology experiments relating to possible adverse effects of 1263W94

Expt	Species	Route	1263W94 concn or dose(s)	Effect
Assessment of the broad pharmacological profile of 1263W94 (PharmaScreen assays)	Mouse, rat, and in vitro	Oral; i.p.[a]	Varied with assay	No gross effects on cardiovascular, gastrointestinal, or central nervous systems; on metabolic parameters; or on microbial activity
Effects on peripheral receptors	Guinea pig and rabbit	In vitro	10 μM	Inhibited responses to acetylcholine and histamine but not to *l*-norepinephrine
Pharmacodynamic effects on central and peripheral nervous systems	Mouse	Oral	250, 500, and 1,000 mg/kg	Overt effects on central and peripheral nervous systems and on respiratory system
Cardiovascular, respiratory, and autonomic effects	Dog	i.v.	0, 3, 10, and 30 mg/kg up to a cumulative dose of 43 mg/kg	Small, statistically significant increases in heart rate, respiratory rate, and respiratory volume; no effect on arterial blood pressure or autonomic function

[a] i.p., intraperitoneal.

TABLE 2. Acute oral and intravenous toxicity studies with 1263W94 in the mouse and rat[a]

Species (strain)	Route	Dose 1263W94 (mg/kg)	No. of mice per group				MNLD (MLD) (mg/kg)
			Total no. dosed		No. that died		
			M	F	M	F	
Mouse (CD-1)	Oral	250	5	5	0	0	M+F = 250 (M = 518; F = 500)
		500	5	5	2	3	
		1,000	5	5	5	5	
Mouse (CD-1)	i.v.	25	1	0	0	ND	M = 25, F = 37.5 (M = 39.3; F = 51.9)
		37.5	5	5	3	0	
		43.75	5	5	2	1	
		50	5	5	5	1	
		62.5	0	5	ND	5	
Rat (Sprague-Dawley)	Oral	1,000	5	5	1	0	M = <1,000, F = 1000 (M = 1092; F = 1250)
		1,500	5	5	5	5	
		2,000	5	5	5	5	
Rat (Sprague-Dawley)	i.v.	50	0	5	ND	0	M+F = 75 (M = 100.9; F = 88.8)
		75	5	5	0	0	
		87.5	0	5	ND	1	
		100	5	5	2	5	
		150	1	0	1	ND	
		175	1	0	1	ND	
		200	5	0	5	ND	

[a] MNLD, maximum nonlethal dose; MLD, median lethal dose; ND, not done; F, female; M, male.

istering single doses of 1263W94 of 10 mg/kg (i.v.) and 10 or 100 mg/kg (oral) to rats (three males/group) and of 10 or 30 mg/kg (oral and i.v.) to monkeys (two animals/sex/group). Blood was collected at various intervals up to 24 h postdose, and the concentrations of 1263W94 in plasma were determined by a reversed-phase high-performance liquid chromatography (HPLC) method described below.

Toxicokinetic data were derived from satellite groups of rats and monkeys in 1-month oral toxicity studies. 1263W94 was administered at doses of 100, 200, and 400 mg/kg/day to rats (three animals/sex/time point/dose). Concentrations in plasma were determined at 0, 2, 5, and 8 h postdose on days 3 and 30. 1263W94 was administered to monkeys (three animals/sex/dose) at doses of 10, 30, and 90 mg/kg twice daily. The concentrations in plasma were determined on days 2 and 27 at 0, 1, 2, 4, and 8 h (just before the second dose) postdose.

Excretion and metabolic profiling of 1263W94 were examined after administration of [^{14}C]1263W94 (rats, 25 μCi/mg; monkeys, 5 μCi/mg) orally and i.v. at 10 mg/kg to rats (three males/dose route) and monkeys (three females/dose route). Animals were housed individually in metabolism cages. Urine and fecal samples were collected from rats at 0 to 24 h, 24 to 48 h, and 48 to 111 h postdose. Urine samples in monkeys were collected at 0 to 8 h, 8 to 24 h, 24 to 48 h, 48 to 72 h, and 72 to 96 h postdose. Feces were collected at 0 to 24 h, 24 to 48 h, 48 to 72 h, and 72 to 96 h postdose. Recovery of radioactivity in urine and fecal extracts was determined with a liquid scintillation counter (Tricarb 1900CA; Packard Instrument Co., Downers Grove, Ill.). The metabolic profile of [^{14}C]1263W94 in urine and fecal extracts was determined by reversed-phase HPLC analysis with radiochemical detection as described below.

As part of a dose range finding study in monkeys (two animals/sex/time point), the concentrations in plasma of putative metabolites of 1263W94 were determined after oral administration of 1263W94 at doses of 10, 30, and 90 mg/kg twice daily for 28 days. Plasma samples were obtained at 0, 1, 2, 4, and 8 h after the first dose on days 3 and 23. As part of the same study, tissue distribution of 1263W94 was determined by obtaining samples of plasma, brain, cerebrospinal fluid, aqueous humor, and vitreous humor from one male and one female

TABLE 3. One-month oral toxicity studies with 1263W94 in the rat and monkey

Species (strain)	Dose of 1263W94 (mg/kg/day)	No. of animals/sex	Effects
Rat (Sprague-Dawley)	0	15[a]	Dose-related, reversible increases (greater severity in females) in neutrophils (≥100 mg/kg/day) and lymphocytes, monocytes, and total WBC counts (≥200 mg/kg/day); mild, reversible, elevations in bilirubin in most females at 200 and 400 mg/kg/day; increases in absolute and relative adrenal, liver, and spleen weights—all of which, except minimal liver weight increases in females at 400 mg/kg/day, were reversible; minimal, reversible, intestinal mucosal hyperplasia at all doses
	100	10[b]	
	200	10[b]	
	400	15[a,b]	
Cynomolgus monkey (*M. fascicularis*)	0	5[c]	Statistically significant differences in reticulocyte count, mean cell hemoglobin concentrations, APTT, and eosinophils between 1263W94 groups and control, which were not considered relevant since they were small in magnitude, occurred during the recovery period, and were not consistently noted during the treatment period; lower absolute and relative adrenal weights without a histopathological correlate
	20[d]	3	
	60[d]	3	
	180[d]	5[c]	

[a] Five animals/sex/group for the recovery period (17 days).
[b] An additional 12 animals were included in these groups for measurement of the levels in plasma only.
[c] Two animals/sex/group for the recovery period (2 weeks).
[d] Administered in divided doses 6 h apart.

monkey 1.5 h after the first dose on day 28. Tissue distribution and the potential for melanin binding were determined by conducting a whole-body autoradiography study in male Sprague-Dawley (albino) or Long Evans (pigmented) rats after oral and i.v. dosing of [^{14}C]1263W94 at 10 mg/kg (50 μCi/rat). After oral dosing, three Sprague-Dawley rats each were euthanized at 0.5, 2, 6, 16, and 48 h and at 7 days postdose, while one Long Evans rat was euthanized at 7 days postdose. Rats receiving the i.v. dose were euthanized at 30 min postdose. Animals were frozen, and sections of various tissues and organs were obtained to quantify [^{14}C]1263W94-derived radioactivity.

1263W94 measurement. An HPLC method specific for the quantitation of 1263W94 and its metabolites was developed and validated. The HPLC system consisted of a Radiomatic A-100 Flo-One/Beta radiochromatography detector (Packard), a WISP712 autoinjector, and a reversed-phase C_{18} (5μ Symmetry) 4.6-by-250-mm column equipped with a precolumn of the same medium (Waters Associates, Milford, Mass.), LDC Constametric 4100 pumps, and an SM5000 UV detector (Laboratory Data Control, Riviera Beach, Fla.). Buffer A consisted of 0.1% triethylamine, adjusted to pH 4.8 with acetic acid and 5% acetonitrile in water. Buffer B was the same as Buffer A, except that it contained 60% acetonitrile. 1263W94 was eluted from the column with a gradient of 40 to 100% Buffer B in Buffer A over 30 min. The flow rate was 1 ml/min; the flow rate of UltimaFlow M liquid scintillation cocktail (Packard) was 2 ml/min. Calibration standards were prepared from dilutions of the dose solution. The peaks were detected by UV absorbance at a 305-nm wavelength.

Human in vitro metabolism. The in vitro metabolism of 1263W94 was studied in microsomal preparations from ten different human livers with characterized cytochrome P450 activities. Human liver microsomes (at a 1-mg/ml protein concentration) obtained from Human Biologics, Inc. (Phoenix, Ariz.) were incubated with 50 μM [^{3}H]1263W94 (14.9 Ci/mmol) at 37°C in phosphate buffer (pH 7.4) containing an NADPH regenerating system. After the reactions were terminated with acetonitrile, the samples were extracted and analyzed by reversed-phase HPLC for 1263W94 and its metabolites. The role of different cytochrome P450 isoforms in the metabolism of 1263W94 was determined by correlation analysis with known cytochrome P450 substrates.

Plasma protein-binding studies. The protein binding of 1263W94 was studied over the concentration range of 0.05 to 200 μg/ml (0.13 to 532 μM) in human plasma and 0.05 to 20 μg/ml (0.13 to 53.2 μM) in monkey, rat, and mouse plasma. [^{14}C]1263W94 (60 mCi/mMol) and unlabeled 1263W94 were directly spiked into the plasma samples and incubated in a 37°C water bath for 15 min. Samples were then dialyzed against 0.142 M phosphate buffer (pH 7.4) for 3 h at 37°C in a Spectrum equilibrium dialyzer with semimicrodialysis cells and Spectra-Por #2 membrane (Spectrum Medical Industries). At the end of dialysis, the radioactivity was determined in plasma and buffer samples, as well as in aliquots of the original spiked plasma samples. In a separate experiment, dissociation constants for 1263W94 binding to human serum albumin, α_1-acid glycoprotein, high-density lipoprotein, and gamma globulin were determined from the fluorescence quenching of the respective proteins, and the kinetics of the binding reaction was investigated by using stopped-flow spectrophotometry.

Safety pharmacology experiments. In the in vitro assay using guinea pig ileum and rabbit aortic tissue, comparisons were made between 1263W94-treated and ethanol vehicle-treated tissues by using two-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple comparisons (SigmaStat for Windows; Jandel Scientific Software, San Rafael, Calif.) at a significance level (P) of <0.05. In the in vivo experiment in dogs, the effects of 1263W94 on blood pressure, heart rate, respiratory rate, and minute volume were compared to the effects of vehicle by using one-way repeated measures ANOVA or Friedman repeated measures ANOVA on ranks if normality test failed (SigmaStat for Windows). In this experiment, pressor/bradycardic responses to bilateral carotid artery occlusion and vagal nerve stimulation, respectively, within treatment groups were compared to their control responses by using Student's paired t test. The level of statistical significance was $P < 0.05$.

Toxicology experiments. In the Ames test and the preincubation modification, a dose-related increase of at least twice the spontaneous revertant colonies by the test material was considered biologically significant, with at least three dose levels showing a dose-related increase in colony numbers. Data from the mouse lymphoma assay were analyzed by the procedure for mammalian fluctuation tests (17) by using Dunnett's test to compare individual doses with the control and a χ^2 analysis of trend. The difference in the log mutant frequencies of the treatment group and control was calculated along with its variance. The test statistic was then computed as the square of the difference divided by the variance of the difference. The test for the linear trend was based on the following model: mutant frequency = spontaneous mutant frequency + dose. The slope, b, and its variance were calculated by using weighted regression. The test statistic was then computed as the square of b divided by its variance. The criteria for a positive response were as follows: (i) demonstration of a statistically significant increase in mutant frequency after treatment with the test substance; (ii) evidence of a dose relationship over at least two dose levels, in any increase in mutant frequency; (iii) demonstration of reproducibility in any increase in dose frequency; and (iv) the observed increases in mutant frequency must lie outside the historical control range with a corresponding day_0 relative survival of ≥10%.

In the micronucleus assay, the number of micronucleated polychromatic erythrocytes (MPCE) was treated as Poisson counts, and the validity of this assumption was determined by a goodness-of-fit test (17). A one-sided version of Shirley's nonparametric test (20) was used to investigate possible decreases in the proportion of polychromatic to normochromatic erythrocytes with dose, as well as to analyze the MPCE counts for male rats 24 h after dosing. In addition, each dose group was also compared with concurrent vehicle controls by means of Wilcoxon's rank sum test by using Bonferroni's correction to adjust the significance level to allow for multiple comparisons.

RESULTS

Safety pharmacology experiments. The results of the PharmaScreen assays showed no gross effects of 1263W94 on the cardiovascular, gastrointestinal, and central nervous systems or on metabolic parameters and microbiological activity. Compared to the vehicle, 1263W94 at 10 μM (3.8 μg/ml) inhibited contractile responses of guinea pig ileum to acetylcholine and histamine but did not affect contractile responses of rabbit aorta to *l*-norepinephrine.

In CD-1 mice, 1263W94 caused hypoactivity, hypothermia, blepharospasm, and a variable respiratory rate at 250 mg/kg; moderate ataxia, coarse tremors, and diarrhea at 500 mg/kg; and loss of traction, coordination, and righting reflexes at 1,000 mg/kg. Lethality, preceded by gasping and cyanosis, was observed in all four high-dose mice within 4 h of dosing. A cumulative i.v. dose of 43 mg of 1263W94/kg in anesthetized dogs had no effect on mean arterial blood pressure or autonomic function but caused small, statistically significant increases in heart rate ($P = 0.02$), respiratory rate ($P = 0.002$), and respiratory volume ($P < 0.001$). The heart rate increased transiently after the 30-mg/kg dose and then decreased to below initial levels. Respiratory rate and minute volume recovered to control values within 40 min of the final dose.

Toxicology experiments. Single oral doses of 1263W94 resulted in lethality at doses ≥500 mg/kg in mice and at 1,500 mg/kg in rats. The no-observed-effect level (NOEL) in mice was 250 mg/kg, whereas minimal toxicity was seen in rats at 1,000 mg/kg (1/10 deaths). The i.v. maximum nonlethal doses were 25 mg/kg (males) and 37.5 mg/kg (females) in mice and 75 mg/kg in both male and female rats.

In the 1-month oral toxicity studies (Table 3), 100 mg/kg/day was the minimally toxic dose in rats (C_{max}, 3.6 to 7.5 μg/ml; area under the concentration-time curve [AUC], 54 to 106 μg · h/ml), and 180 mg/kg/day was the NOEL in monkeys (C_{max}, 6.4 to 6.9 μg/ml; AUC, ca. 30 μg · h/ml). The specific findings are presented in Table 3.

In mutagenicity studies, 1263W94 was not mutagenic at concentrations of ≤650 μg/plate in the Ames test and at concentrations of ≤205 μg/plate in the preincubation modification bacterial mutation assay in the absence or presence of metabolic activation. In four tests carried out with the mouse lymphoma assay, 1263W94 was mutagenic in the absence of an in vitro metabolic activation system (i.e., rat liver microsomal S-9 mix) and equivocal in the presence of S-9 mix (Table 4). In two tests carried out in the absence of metabolic activation, statis-

TABLE 4. Mouse lymphoma mutagenesis assay of 1263W94[a]

Concn (μg/ml) of 1263W94	Results in the absence of S-9 mix				Results in the presence of S-9 mix			
	Test 1		Test 2		Test 1		Test 2	
	Mean mutant frequency (10^4)	Mean survival (%)	Mean mutant frequency (10^4)	Mean survival (%)	Mean mutant frequency (10^4)	Mean survival (%)	Mean mutant frequency (10^4)	Mean survival (%)
0	2.1	100	2.3	100	2.0	100	2.8	100
5	2.3	83	2.9	112	ND	95	1.5	103
10	3.1	71	2.3	70	ND	84	2.5	74
15	3.5[b]	48	2.5	72	ND	ND	ND	ND
20	4.0[b]	27	6.6[b]	37	1.5	80	ND	ND
25	5.1[b]	16	6.9[a]	21	1.9	68	2.3	70
50	4.2[b]	9	9.0[b]	7	1.8	59	3.0	50
75	5.6[b]	3	ND	3	4.0[b]	56	1.9	42
100	ND	ND	ND	ND	3.0	53	ND	36
150	NA	0	NA	0	3.4	25	ND	6
Positive control[c]	1.3[b]	54	1.1[b]	68	1.2[b]	41	6.6[b]	10

[a] Values for the vehicle control are the mean of four measurements; all others are the mean of two measurements. ND, not determined. NA, not applicable.
[b] Statistically different from control (1263W94 = 0) with $P < 0.01$.
[c] The positive control for tests in the absence of S-9 mix was methylmethanesulfonate; in the presence of S-9 mix, the positive control was 20-methylcholanthrene.

tically significant dose-related increases in mutant frequencies were observed at doses ranging from 15 to 75 μg/ml (test 1) and from 20 to 75 μg/ml (test 2). Methylmethanesulfonate, used for the positive control, induced highly significant increases in mutant frequency in these tests.

Two mouse lymphoma tests were carried out in the presence of a metabolic activation system (Table 4). Statistically significant increases in mutant frequency were observed at 75 and 150 μg/ml (test 1) in the presence of S-9 mix, but the increases were not dose related. No statistically significant increases in mutant frequency were observed in test 2. Therefore, no conclusive evidence of mutagenic potential was demonstrated in the presence of S-9 mix, and the mutagenicity of the compound is considered equivocal in the presence of S9. 20-Methylcholanthrene, used for the positive control, induced highly significant increases in mutant frequency in these tests. It was concluded that 1263W94 demonstrated mutagenic potential in this mouse lymphoma mammalian cell mutation assay in the absence of S9.

1263W94 was not clastogenic in an in vivo micronucleus assay at oral doses of ≤1,200 mg/kg in rats. The highest dose tested in this assay produced a total 1263W94 plasma level of ca. 11 μg/ml.

Pharmacokinetics and toxicokinetics of 1263W94. The mean pharmacokinetic parameters for 1263W94 in rats and monkeys are presented in Table 5. Some evidence of enterohepatic recirculation was observed in both species. As a result, terminal half-life in plasma could not be determined. Oral bioavailability was high in the rat (88 to 92%) and variable in the monkey (42 to 58%).

Biliary excretion was the major pathway of clearance in the rat after i.v. or oral doses of [^{14}C]1263W94 at 10 mg/kg, with 89.7 and 89.2% of the doses, respectively, eliminated in the feces and 94.2 and 91.3% of the doses, respectively, recovered in the excreta as unchanged drug. The recovery of radiolabel in the urine averaged 6.1 and 5.4% of the dose, respectively. Biliary excretion was a significant route of elimination in the monkey, with 41.0 and 35.6% of the dose eliminated in the feces and 57.5 and 36.8% of the dose recovered as unchanged drug after i.v. and oral dosing, respectively. The recovery of the radiolabel in the urine averaged 15.9 and 17.4%, respectively.

The N-dealkylated analog of 1263W94 was excreted in the

TABLE 5. Mean pharmacokinetic parameters in rats and monkeys dosed with 1263W94

Species (strain)	Route	No. and sex of animals[c]	Dose (mg/kg) of 1263W94	1263W94[a]						
				AUC (h · μg/ml)	$t_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	CL (liters/kg/h)	V (liters/kg)	F (%)
Rat (Sprague-Dawley)	Oral	3M	10	5.16	NC[b]	1.29	6.5	NC	NC	92
	Oral	3M	100	49.48	NC	4.94	1.0	NC	NC	88
	i.v.	3M	10	5.63	0.38			1.80	1.61	
Cynomolgus monkey (*M. fascicularis*)	Oral	2M, 2F	10	9.1	NC	1.15	1.38	1.66	NC	58
	Oral	2M, 2F	30	17.3	NC	3.20	1.75	2.05	NC	42
	i.v.	2M, 2F	10	14.7	NC			0.79	21.6	
	i.v.	2M, 2F	30	40.1	NC			0.76	19.0	

[a] AUC, AUC_{0-24} in rats and $AUC_{0-\infty}$ in monkeys; CL, clearance; V, volume of distribution; F, oral bioavailability.
[b] NC, not calculated. Due to the slow rate of elimination due to enterohepatic circulation, elimination-phase half-lives in plasma could not be determined from the data collected.
[c] M, male; F, female.

TABLE 6. Toxicokinetic parameters in rats and monkeys after receiving oral doses of 1263W94 for 1 month

Species (time)	Dose (mg/kg/day)	Sex[b]	C_{max} (μg/ml)		AUC (h · μg/ml)	
			Total	Free[a]	Total	Free[a]
Rat (day 30, 0–24 h)	100	M	3.6	0.43	54.3	6.52
		F	7.5	0.90	106	12.7
	200	M	8.1	0.97	104	12.5
		F	17.0	2.04	200	24.0
	400	M	9.7	1.16	157	18.8
		F	21.6	2.59	349	41.9
Monkey (day 27, 0–8 h)	20	M	0.9	0.14	4.6	0.74
		F	0.7	0.11	4.5	0.72
	60	M	2.6	0.42	12.8	2.05
		F	2.2	0.35	12.3	1.97
	180	M	6.9	1.10	30.6	4.90
		F	6.4	1.02	29.2	4.67

[a] All estimates of free drug concentrations were calculated based on the in vitro protein-binding measurements (88% in rats and 84% in monkeys).
[b] M, male; F, female.

urine and feces of rats (5.3% of the i.v. dose and 5.1% of the oral dose) and was also identified in the urine (4.6% of the i.v. dose and 6.6% of the oral dose) and feces (11.4% of the i.v. dose and 15.5% of the oral dose) of monkeys. In monkeys given 1263W94 orally at 10, 30, or 60 mg/kg twice daily for 28 days, the N-dealkylated metabolite was also identified as a putative plasma metabolite. Mean peak levels in plasma increased with dose, and the average $AUC_{0\text{-}8}$ for the metabolite was 8 to 14% of the corresponding $AUC_{0\text{-}8}$ for the parent drug regardless of the dose.

In the 30-day toxicokinetic studies (Table 6), systemic exposure to orally administered 1263W94 was up to twofold higher in female rats, but no significant sex differences were apparent in monkeys. AUC values were proportional to doses of up to 200 mg/kg/day (middle dose) in rats and 180 mg/kg/day (highest dose) in monkeys.

After i.v. or oral doses of [^{14}C]1263W94 (50 μCi/rat) to albino and pigmented rats, radioactivity was widely distributed, with the highest levels found in the gastrointestinal tract. High levels of radioactivity in the gastrointestinal tract on the seventh day postdose was indicative of delayed excretion possibly due to enterohepatic recirculation. Biliary excretion was confirmed by high levels of radioactivity in the small intestine after i.v. dosing. Moderate levels of radioactivity in the uveal tract and hair follicles of pigmented animals demonstrated evidence of melanin binding. The absence of radioactivity in the brain and spinal cord indicated poor penetration of 1263W94 across the blood-brain barrier in this species. 1263W94 was able to penetrate into brain tissue in monkeys given 10, 30, or 90 mg/kg twice daily for 28 days. On day 28, the concentrations of 1263W94 in plasma ranged from 1 to 13 μg/ml. 1263W94 was not quantifiable in samples of aqueous humor (<0.09 μg/ml), but it was detectable in the brain (4 to 20% of 1263W94 levels measured in plasma), cerebrospinal fluid (1 to 2% of 1263W94 plasma levels), and vitreous humor (<1% of plasma 1263W94 levels), with the levels in these tissues generally paralleling corresponding 1263W94 levels in plasma.

Human in vitro metabolism. If two reactions are catalyzed by the same enzyme or family of enzymes, the rates of formation should correlate to each other. Ten different human liver microsomal preparations with characterized cytochrome P450 activities were used to metabolize 1263W94 to its N-dealkylated metabolite and its base, the aglycone. The role of different P450 isoforms in the metabolism of 1263W94 was determined by correlation analysis with known cytochrome P450 substrates. Strong correlations were observed between the rate of formation of the N-dealkylated metabolite (r^2 = 0.97), testosterone 6β-hydroxylation (a marker for CYP3A4 activity), and cytochrome P450 content (r^2 = 0.83). These results suggest that the formation of the N-dealkylated metabolite of 1263W94 is catalyzed by isozymes within the CYP3A subfamily. The rate of formation of the aglycone did not correlate with either 6β-hydroxylation of testosterone or cytochrome P450 content. No significant correlations were observed between rates of N-dealkylated metabolite or aglycone formation with markers for CYP1A1, 1A2, 2A6, 2B6, 2D6, 2C9, 2C19, 2E1, or 4A9/11.

Plasma protein binding of 1263W94. Protein binding was determined by equilibrium dialysis over the concentration ranges of 0.05 to 200 μg/ml (0.13 to 532 μM) in human plasma and 0.05 to 20 μg/ml (0.13 to 53 μM) in monkey, rat, and mouse plasma. The binding of 1263W94 was very extensive in human plasma and somewhat lower in monkey, rat, and mouse plasma. Protein binding of 1263W94 in all species appeared to decrease slightly with increasing concentrations over the range tested. The average binding of 1263W94 was 98.0% in human plasma, 83.9% in monkey plasma, 88.1% in rat plasma, and 84.7% in mouse plasma.

The dissociation constants for 1263W94 binding to various blood proteins measured by fluorescence quenching were as follows: human serum albumin (K_d = 63 μM), α_1-acid glycoprotein (K_d = 9.4 μM), high-density lipoprotein (K_d = 80 μM), and gamma globulin (K_d = ~800 μM). Although the binding affinity for α_1-acid glycoprotein is somewhat higher than that for serum albumin, serum albumin accounts for the majority of proteins bound to 1263W94 since it is present at higher concentrations in human plasma.

DISCUSSION

HCMV is a prime opportunistic pathogen among immunosuppressed individuals and neonates. In immunocompromised hosts, such as transplant recipients and AIDS patients, HCMV infection requires prolonged courses of suppressive therapy. Of the drugs currently approved in the United States for the treatment of HCMV infection, only GCV and the prodrug valganciclovir are approved for use in transplant recipients, and GCV therapy is often poorly tolerated in these patients. The effectiveness of existing therapies for HCMV disease is generally limited by drug toxicities, including hematologic disorders such as granulocytopenia, anemia, and thrombocytopenia, and renal impairment. There is a need for a safe and effective oral therapy that can be used for prevention and/or treatment of HCMV disease in immunocompromised individuals and neonates and for prevention and/or treatment of reactivation of latent HCMV infection in immunocompromised individuals. The preclinical profile presented here suggests that 1263W94 could meet these criteria.

Assessment of the broad pharmacological profile of central

nervous system, cardiovascular, metabolic, gastrointestinal, and microbiological activity of 1263W94 showed no significant activity at doses ranging from 10 to 300 mg/kg in vivo and at concentrations ranging from 3 to 30 μg/ml in vitro. Although 1263W94 (10 μM) produced anticholinergic and antihistaminergic effects in vitro, there was no evidence of in vivo antagonism of these transmitters at oral doses of 100 mg/kg. In vivo studies at relatively high doses produced central and peripheral nervous system and respiratory effects in mice (250 to 1,000 mg/kg given orally) and produced mild cardiovascular and respiratory effects in dogs (cumulative i.v. dose of 43 mg/kg).

In acute toxicity studies, administration of 1263W94 was well tolerated in mice and rats at oral doses of 250 and 1,000 mg/kg, respectively. The clinical signs seen with larger doses in mice and rats were nonspecific. Limited toxicity was observed in the 1-month toxicity studies in rats and monkeys. In rats, treatment-related findings were reversible except for minimal increases in liver weights, absolute neutrophils, and absolute monocytes in the high dose (400 mg/kg/day) females only. No significant treatment-related changes were observed in monkeys in these acute studies. Chronic toxicology studies have subsequently been carried out, and the results continue to support a favorable safety profile for this clinical candidate (GlaxoSmithKline [data on file]). The toxicology profile for GCV includes reports of aspermatogenesis, mutagenicity, teratogenicity, carcinogenicity, and bone marrow depression (9). The clinical dose-limiting toxicities of GCV are granulocytopenia, anemia, and thrombocytopenia. In animal modeling of transplant regimens, GCV doses of 5 mg/kg/day were associated with delayed platelet recovery in toxicology studies in dogs receiving i.v. administration of ganciclovir prophylactically after whole-body irradiation and autologous bone marrow transplantation (2). More severe toxicities have been noted in animal studies with CDV. In a 26-week toxicology study, female rats receiving weekly subcutaneous injections of CDV developed mammary adenocarcinomas at CDV doses of 0.6 mg/kg/week (a dose ca. 0.04-fold the systemic exposure in humans at the recommended i.v. dose), leading to premature termination of the study (Vistide package insert; Gilead Sciences, Inc.).

Results from mutagenicity studies with 1263W94 indicate that the drug is unlikely to represent a genotoxic hazard to humans. 1263W94 was not mutagenic at concentrations of ≤650 μg/plate in the standard Ames test and at concentrations of ≤205 μg/ml in the preincubation assay with or without metabolic activation. GCV and CDV were also not mutagenic in the Ames test.

1263W94 (15 to 75 μg/ml) demonstrated mutagenic potential in the in vitro mouse lymphoma assay in the absence of metabolic activation. However, no conclusive evidence of mutagenic potential was observed in the presence of metabolic activation. The mechanism for the increase in mutant frequency in the mouse lymphoma assay is unknown but is unlikely to involve phosphorylation and subsequent incorporation into mammalian DNA. 1263W94 is not phosphorylated to a detectable degree in vitro, and it does not inhibit incorporation of deoxyribonucleoside triphosphates by human DNA polymerase (3). 1263W94 was not clastogenic in the rat bone marrow micronucleus assay, 24 and 48 h after oral administration, at doses of 400 to 1,200 mg/kg (body weight). The highest dose level produced a total 1263W94 plasma level of ca. 11 μg/ml. These results contrast with those seen with GCV, which was clastogenic in the mouse micronucleus assay at doses ca. 2.8-fold (150 mg/kg) to 10-fold (500 mg/kg) larger than the dose given in human exposure (Cytovene package insert; Roche Pharmaceuticals). In addition, GCV showed a potent dose-dependent activity in clastogenicity assays in Chinese hamster V79-E cells at concentrations of $>2.2 \times 10^{-4}$ M (19).

The pharmacokinetic profiles of 1263W94 in both rodents and primate species support its potential as an oral agent. 1263W94 was well absorbed in rats and monkeys and was primarily excreted unchanged in the feces. Excellent oral bioavailability was demonstrated in both species, with oral doses of 10 mg/kg resulting in concentrations in plasma that were 24- to 114-fold greater than the mean IC_{50} for 10 HCMV clinical isolates. The elimination half-life in rats could not be determined because of a prolonged absorption phase, and estimates of the half-life in monkeys were complicated by evidence of enterohepatic recirculation. Biliary excretion appeared to be the major pathway for clearance in the rat and a predominant pathway in the monkey. N dealkylation of 1263W94 appeared to be the predominant metabolic pathway in both species. The results from in vitro studies suggest that CYP3A4 is most likely the major cytochrome P450 isozyme responsible for the metabolism of 1263W94 to its N-dealkylated analog in humans.

In 30-day oral toxicity studies in rats and monkeys, mean peak concentrations and exposures to 1263W94 increased in near proportion to dose. 1263W94 was able to penetrate the brain, cerebrospinal fluid, and vitreous humor of cynomolgus monkeys, but levels in these tissues were low. 1263W94 is highly bound to human plasma proteins, with serum albumin being the predominant binding site; however, a high dissociation constant indicates that this binding is readily reversible. Relative to humans, there was less binding of 1263W94 to monkey, rat, and mouse plasma proteins.

1263W94 is a potent inhibitor of HCMV and is currently being evaluated in Phase I/II clinical trials. The results of the preclinical studies with 1263W94 presented here suggest that it is a safe agent for the treatment of HCMV disease. With the favorable safety profile demonstrated in these preclinical studies, good oral bioavailability, and lower toxicity than currently available anti-HCMV agents, 1263W94 has the potential to be a viable treatment option for HCMV disease.

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REFERENCES

1. **Ames, B., F. Lee, and W. Durston.** 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA **70:**782–786.
2. **Applebaum, F., J. Meyers, H. Deeg, T. Graham, F. Schuening, and R. Storb.** 1988. Toxicity trial of prophylactic 9-[2-hydroxy-1-(hydroxymethyl)ethyoxymethyl]guanine (ganciclovir) after marrow transplantation in dogs. Antimicrob. Agents Chemother. **32:**271–273.
3. **Biron, K. K., R. G. Harvey, S. C. Chamberlain, S. S. Good, A. A. Smith III, M. G. Davis, C. L. Talarico, W. H. Miller, R. Ferris, R. E. Dornsife, S. C. Stanat, J. C. Drach, L. B. Townsend, and G. W. Koszalka.** 2002. Potent and

selective inhibition of human cytomegalovirus replication by 1263W94, a benzimidazole L-riboside with a unique mode of action. Antimicrob. Agents Chemother. **46:**2365–2372.
4. **Chou, S.** 1999. Antiviral drug resistance in human cytomegalovirus. Transpl. Infect. Dis. **1:**105–114.
5. **Chrisp, P., and S. Clissold.** 1991. Foscarnet. Drugs **41:**104–129.
6. **Chulay, J., K. Biron, L. Want, M. Underwood, S. Chamberlain, L. Frick, S. Good, M. Davis, R. Harvey, L. Townsend, J. Drach, and G. Koszalka.** 1999. Development of novel benzimdazole riboside compounds for treatment of cytomegalovirus disease, p. 129–134. *In* J. Mills, P. Volberding, and L. Corey (ed.), Antiviral therapy 5: new directions for clinical applications and research. Kluwer Academic/Plenum Publishers, New York, N.Y.
7. **Clive, D., and J.-A. Spector.** 1975. Laboratory procedure for assessing specific locus mutations in cultured L5178Y mouse lymphoma cells. Mutat. Res. **31:**17–29.
8. **Drew, W., D. Ives, J. Lalezari, C. Crumpacker, S. Follansbee, S. Spector, C. Benson, D. Friedberg, L. Hubbard, M. Stempien, et al.** 1995. Oral ganciclovir as maintenence treatment for cytomegalovirus retinitis in patients with AIDS. N. Engl. J. Med. **333:**615–620.
9. **Faulds, D., and R. Heel.** 1990. Ganciclovir: a review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs **39:**597–638.
10. **Griffiths, P.** 2002. The treatment of cytomegalovirus infection. J. Antimicrob. Chemother. **49:**243–253.
11. **Hayashi, M., R. Tice, J. MacGregor, D. Anderson, D. Blakey, M. Kirsch-Volders, F. Oleson, Jr., F. Pacchierotti, F. Romagna, H. Shimada, S. Sutou, and B. Vannier.** 1994. In vivo rodent erythrocyte micronucleus assay. Mutat. Res. **312:**293–304.
12. **Jabs, D., C. Enger, J. Dunn, and M. Forman.** 1998. Cytomegalovirus retinitis and viral resistance: ganciclovir resistance. J. Infect. Dis. **177:**770–773.
13. **Jabs, D., C. Enger, M. Forman, and J. Dunn.** 1998. Incidence of foscarnet resistance and cidofovir resistance in patients treated for cytomeglovirus retinitis. Antimicrob. Agents Chemother. **42:**2240–2244.
14. **Lalezari, J., W. Drew, E. Glutzer, C. James, D. Miner, J. Flaherty, P. Fisher, K. Cundy, J. Hannigan, and J. Martin.** 1995. (*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir): results of a phase I/II study of a novel antiviral nucleotide analog. J. Infect. Dis. **171:**788–796.
15. **Lalezari, J., W. Drew, E. Glutzer, C. James, D. Miner, S. Safrin, W. Owen, J. Davidson, P. Fisher, and H. Jaffe.** 1994. Treatment with intravenous (*S*)-1-[3-hydroxy-2-(phosphonyl-methoxy)propyl]cytosine of acyclovir-resistant mucocutaneous infection with herpes simplex virus in a patient with AIDS. J. Infect. Dis. **170:**570–572.
16. **Maron, D., and B. Ames.** 1983. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res. **113:**173–215.
17. **Robinson, W., M. Green, J. Cole, R. Garner, M. Healy, and D. Gatehouse.** 1989. Statiscal evaluation of bacterial/mammalian fluctuation tests, p. 102–108. *In* D. Kirkland (ed.), UKEMS Subcommittee on guidelines for mutagenicity testing. III. Statistical evaluation of mutagenicity test data. Cambridge University Press, Cambridge, England.
18. **Spector, S., D. Busch, S. Follansbee, K. Squires, J. Lalezari, M. Jacobson, J. Connor, D. Jung, A. Shadman, and B. Master.** 1995. Pharmacokinetic, safety and antiviral profiles of oral ganciclovir in persons infected with human immunodeficiency virus: a phase I/II study. J. Infect. Dis. **171:**1431–1437.
19. **Thust, R., M. Schacke, and P. Wutzler.** 1996. Cytogenetic genotoxicity of antiherpes virostatics in Chinese hamster V79-E cells. I. Purine nucleoside analogues. Antivir. Res. **31:**105–113.
20. **Williams, D.** 1986. A note on Shirley's non-parametric test for comparing several dose levels with a zero-dose control. Biometrics **42:**183–186.