TOXICOLOGICAL SCIENCES 44, 97–106 (1998)
ARTICLE NO. TX982481

Effect of Oral Probenecid Coadministration on the Chronic Toxicity and Pharmacokinetics of Intravenous Cidofovir in Cynomolgus Monkeys

Steven A. Lacy,*,[1] Michael J. M. Hitchcock,* William A. Lee,* Pierre Tellier,† and Kenneth C. Cundy*

*Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, California 94404; and †ClinTrials BioResearch Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, Canada H9X 3R3

Received October 17, 1997; accepted May 1, 1998

Effect of Oral Probenecid Coadministration on the Chronic Toxicity and Pharmacokinetics of Intravenous Cidofovir in Cynomolgus Monkeys. Lacy, S. A., Hitchcock, M. J. M., Lee, W. A., Tellier, P., and Cundy, K. C. (1998). *Toxicol. Sci.* 44, 97–106.

In animals and humans, intravenous administration of the antiviral nucleotide analogue cidofovir results in a dose-limiting nephrotoxicity characterized by damage to the proximal tubular epithelial cells. Probenecid, a competitive inhibitor of organic anion transport in the proximal tubular epithelial cells, was evaluated for its effect on the chronic toxicity and pharmacokinetics of cidofovir. Cynomolgus monkeys (5/sex/group) received cidofovir for 52 consecutive weeks as a once weekly intravenous bolus injection at 0 (saline), 0.1, 0.5, or 2.5 mg/kg/dose alone or at 2.5 mg/kg/dose in combination with probenecid (30 mg/kg/dose via oral gavage 1 h prior to cidofovir administration). Cidofovir-associated histopathological changes were seen only in the kidneys, testes, and epididymides. Nephrotoxicity (mild to moderate cortical tubular epithelial cell karyomegaly, tubular dilatation, basement membrane thickening) was present only in monkeys receiving 2.5 mg/kg/dose cidofovir without probenecid. The incidence and severity of testicular (hypo- and aspermatogenesis) and epididymal (severe oligo- and aspermia) changes were increased in monkeys administered cidofovir at 2.5 mg/kg/dose, either alone or in combination with oral probenecid. Renal drug clearance was decreased between Weeks 1 and 52 in the 2.5 mg/kg/dose groups and resulted in an increased systemic exposure to cidofovir (as measured by AUC) that was significantly greater in monkeys administered cidofovir alone (312% increase in males, 98% in females) than in those coadministered probenecid (32% increase in males, 3% in females). These results demonstrate that oral probenecid coadministration protects against the morphological evidence of nephrotoxicity and the accompanying decrease in renal clearance in monkeys receiving chronic intravenous cidofovir treatment.

Key Words: 1-[(*S*)-3-hydroxy-2-phosphonomethoxy)propyl]cytosine; Cidofovir; Cidofovir injection (Vistide); biological activity; nucleotide analogue with antiherpesvirus activity

Cidofovir (1-[(*S*)-3-hydroxy-2-phosphonomethoxy)propyl]-cytosine) is a cytosine nucleotide analogue with potent *in vitro* and *in vivo* activity against a broad spectrum of herpesviruses, including many linked to human diseases (for recent reviews see Hitchcock *et al.*, 1996; Naesens *et al.*, 1997). Cidofovir injection (Vistide) was recently approved in the United States and Europe for the treatment of cytomegalovirus retinitis in patients with AIDS. Cellular enzymes convert cidofovir to several phosphorylated metabolites, including cidofovir diphosphate, the putative antiviral species and potent inhibitor of herpes DNA polymerases. The long intracellular half-lives observed *in vitro* (Ho *et al.*, 1992; Cihlar *et al.*, 1992) for the phosphorylated metabolites (cidofovir diphosphate $t_{\frac{1}{2}}$ = 17–65 h) permit an infrequent clinical dosing schedule of once every 2 weeks for cidofovir injection maintenance therapy.

Nephrotoxicity is the dose-limiting toxicity for systemically administered cidofovir in all species examined thus far, including humans (Lalezari *et al.*, 1995, 1997; SOCA, 1997). In animal studies, cidofovir-associated kidney toxicity affects the proximal tubular epithelial cells of the renal cortex. Lesions may range from cytomegaly and karyomegaly of individual cells to degeneration, necrosis, and regeneration of the renal cortical tubular epithelium. Additional cidofovir-associated target tissues observed in animal toxicity studies include bone marrow (erythroid and myeloid depletion), testes (hypospermia), and, to a lesser degree, spleen and thymus (lymphoid depletion).

Cidofovir administered intravenously to rabbits, monkeys, or humans shows generally dose-proportional pharmacokinetics, with the majority (> 80%) of the administered dose excreted in the urine by 24 h as unchanged drug (Cundy *et al.*, 1994, 1995, 1996a). Renal clearance of cidofovir in these species exceeds the corresponding glomerular filtration rate, indicating active tubular secretion of the drug by the kidney. [^{14}C]Cidofovir was also shown to concentrate preferentially in monkey kidney at levels > 1000-fold higher than plasma levels by 120 h postdose. Accumulation of cidofovir in the kidney is thought to result from active uptake from blood by the organic anion transporter on the antiluminal membrane of the proximal

[1] To whom correspondence should be addressed at present address: Pharmacyclics, Inc., 995 East Arques Ave., Sunnyvale, CA 94086.

1096-6080/98 $25.00

tubular epithelium and a slower rate of efflux of drug into the tubular lumen. This selective uptake and long intracellular half-life of drug in the proximal tubular epithelial cells appears to underlie cidofovir-associated nephrotoxicity.

Coadministration of probenecid, a competitive inhibitor of organic anion transport in the kidney, has been used clinically to decrease plasma clearance rates of selected acidic drugs, chiefly antibiotics, that undergo active tubular secretion (Cunningham *et al.*, 1981). Pilot studies in rabbits with radiolabeled intravenous cidofovir demonstrated that concomitant oral probenecid administration decreased initial drug levels in kidney cortex, but had no effect on drug levels in other tissues (Cundy *et al.*, 1994, 1996b). Subsequent repeat dose toxicity studies showed the nephrotoxicity resulting from intravenous cidofovir (25 mg/kg/day daily for 5 days) in rabbits could be completely prevented by probenecid (iv split doses 15 min predose and 4 h postdose) at a 6:1 probenecid:cidofovir ratio and partially ameliorated at a 2:1 ratio. Likewise, an oral probenecid regimen (30 mg/kg po 1 h predose) decreased the severity of nephrotoxicity in cynomolgus monkeys dosed with intravenous cidofovir (5 mg/kg/dose) once weekly for 13 weeks (Lacy and Hitchcock, unpublished data). In these studies, probenecid appeared to decrease kidney tissue drug levels and associated nephrotoxicity by acting as a competitive inhibitor of cidofovir uptake by the proximal tubular epithelial cells.

Probenecid is currently used as a nephroprotectant during clinical therapy with intravenous cidofovir. In HIV-infected patients, concomitant oral probenecid (administered as 2 g given 3 h prior to cidofovir infusion and 1 g each 2 and 8 h after the end of the cidofovir infusion) decreased the renal clearance of a 5 mg/kg cidofovir dose to a level consistent with glomerular filtration, suggesting that it had effectively blocked the active tubular secretion of cidofovir (Cundy *et al.*, 1995). Concomitant oral probenecid also prevented the increase in serum creatinine levels observed in HIV patients receiving intravenous cidofovir alone, indicating a nephroprotective effect (Lalezari *et al.*, 1995). The objective of the present study was to characterize the effect of a clinically relevant oral probenecid dosing regimen (30 mg/kg 1 h prior to cidofovir injection) on the chronic toxicity and pharmacokinetics of intravenous cidofovir in cynomologus monkeys dosed once weekly for 52 consecutive weeks. This dosing regimen for probenecid was derived empirically, with the rationale being to maximize probenecid exposure around the time of peak cidofovir exposure to the kidneys to minimize the tubular secretion of cidofovir.

MATERIALS AND METHODS

Chemicals. Cidofovir (98.2% purity) was supplied by Gilead Sciences, Inc. as a sterile 75 mg/ml solution. Probenecid (> 99% purity) was purchased from Sigma Chemical Company (St. Louis, MO). Sterile Saline for Injection U.S.P. and Sterile Water for Injection U.S.P. were purchased from Baxter, Inc. (Toronto, Ontario), Abbott Laboratories Ltd (Montreal, Quebec), or Clintec Nutrition Co. (Toronto, Ontario). Chemicals were stored at room temperature and out of direct light when not in use. During the treatment phase, dosing formulations of cidofovir (in Sterile Saline) and probenecid (in Sterile Water) were prepared once weekly and stored refrigerated (ca. 4°C) until used.

Stability and potency of cidofovir dosing formulations were verified using a validated isocratic, ion-pairing HPLC method. The chromatographic conditions are mobile phase (5.0 mM tetrabutylammonium dihydrogen phosphate, 3.5 mM sodium phosphate, pH 5.9); 2 ml/min; 20 μl sample injection volume; 25°C; and 20-min run time. Cidofovir (peak retention time, 6–10 min) and its primary degradation product 1-[3-hydroxy-2-(phosphonomethoxy)propyl]uridine (peak retention time, 8–13.5 min) are detected via UV absorption (280 nm) and measured quantitatively by calculation of relative peak area using external reference standards. The validated method has been shown to have an accuracy of 99.3 ± 0.4%, a repeatability of 0.4%, and a precision of 99.3 ± 0.4 to 110.3 ± 0.3%.

Animals. Young adult cynomolgus monkeys (41/sex) were purchased from HRP, Inc. (Denver, PA). Animals were housed and treated at ClinTrials BioResearch Laboratories (Senneville, Quebec). Body weight ranges taken 1 or 2 days prior to treatment initiation were 2.5 to 3.4 kg (males) and 2.5 to 3.6 kg (females). Each animal was uniquely identified by a permanent skin tattoo on the inner thigh. Prior to initiation of treatment, all animals were acclimated 42 (males) or 43 (females) days, during which time they were tested three times and shown to be negative for tuberculosis. Environmental conditions were monitored and controlled (temperature: 24 ± 3°C; humidity: 50 ± 20%; illumination: 12 h light/12 h dark). A standard certified commercial laboratory diet (PMI Primate Laboratory Chow) was supplied to each monkey (approximately 200 g/day). Tap water (treated by reverse osmosis and sterilized by ultraviolet light) was available *ad libitum*.

Dose selection rationale and administration. Cidofovir doses used in the present study (0.1, 0.5, and 2.5 mg/kg) were selected based on results from a previous study in which kidney lesions were observed histopathologically in cynomolgus monkeys administered cidofovir intravenously (5 mg/kg/dose) once weekly for 13 weeks. The severity of these kidney lesions was decreased in animals coadministered oral probenecid (30 mg/kg/dose 1 h prior to iv cidofovir). This probenecid dosage was based on achieving a dose comparable (on a mg/kg basis) to the initial dose (2 g po 3 h prior to iv cidofovir infusion) that is being used clinically. In phase I/II clinical trials, oral probenecid cotreatment, in conjunction with saline prehydration and an extended dosing duration, reduced the incidence of proteinuria and increased serum creatinine that had been reported when using cidofovir without probenecid (Lalezari *et al.*, 1995). Previously conducted clinical pharmacokinetic studies of cefoxitin, a cephalosporin eliminated by renal tubular secretion, had also shown that a 2-g dose of probenecid administered per os 1 h prior to intramuscular cefoxitin resulted in AUC values greater than that achieved by either a 1-g dose of probenecid given 1 h before cefoxitin or a 1-g dose of probenecid coadministered with cefoxitin (Vlasses *et al.*, 1980).

The experimental design for this study is found in Table 1. Cidofovir injections (1 ml/kg) were administered via intravenous bolus through a 23- or 25-gauge needle and were rotated among four separate administration sites (two saphenous veins and two cephalic veins). All animals received either probenecid or Sterile Water via oral gavage (3 ml/kg) approximately 1 h prior to cidofovir administration, followed by a 5 ml Sterile Water washout of the rubber gavage tube. This study followed current Good Laboratory Practice Guidelines of the U.S. Food and Drug Administration.

In-life observations. Animals were observed daily for clinical signs of toxicity. Body weights were evaluated weekly and food consumption was evaluated daily. Ophthalmoscopic evaluations were performed prior to treatment initiation and at study termination. Clinical chemistry and hematology evaluations were conducted prior to treatment initiation, during Weeks 4, 13, and 26 (6-month cohort) or Weeks 3, 12, 25, and 38, and at termination (12-month cohort). Serum blood urea nitrogen and creatinine were measured quantitatively using a Hitachi 717 analyzer. Urinalysis was done prior to treatment and during Weeks 3, 12, and 25 (6-month cohort) or Weeks 24 and 50 (12-month cohort). Urinary protein and glucose were measured semiquantitatively using validated dipstick methods.

TABLE 1
Tabulated Summary of Chronic Toxicity in Cynomolgus Monkeys Administered Intravenous Cidofovir in the Presence or Absence of Coadministered Oral Probenecid

Group	No. of animals/ sex/group	Dose level[a] (mg/kg/dose)		Major test article-related findings
		Cidofovir	Probenecid	
1	5	0	0	None
Recovery	2	0	0	None
26-Week cohort	5	0	0	None
2	5	0.1	0	None
3	5	0.5	0	None
4	5	2.5	0	Increased BUN, creatinine; glucosuria; proteinuria; renal tubular epithelial cell karyomegaly, tubular dilatation, basement membrane thickening (mild–moderate); testicular hypo/aspermatogenesis, secondary epididymal oligo/aspermia (moderate–severe)
Recovery	2	2.5	0	Comparable histopathologic severity and incidence as above
5	5	2.5	30	Testicular hypo/aspermatogenesis, secondary epididymal oligo/ aspermia (moderate–severe)
Recovery	2	2.5	30	Comparable histopathologic severity and incidence as above
26-Week cohort	5	2.5	30	None

[a] Cidofovir was administered as a single intravenous bolus injection once weekly for 26 consecutive weeks (5/sex/Groups 1 and 5) or 52 consecutive weeks (5/sex/Groups 1–5). Probenecid was administered as a single oral gavage dose 1 h prior to cidofovir treatment. Groups 1, 4, and 5 Recovery cohorts (2/sex/group) had a 4-week postdose, nontreatment phase following 52 dosing weeks to evaluate reversibility of cidofovir-associated lesions.

Observations at necropsy. On completion of the treatment and observation periods, animals were food-fasted overnight, administered ketamine hydrochloride for injection U.S.P. (10 mg/kg im), then euthanized with sodium pentobarbital followed by exsanguination by incision of the axillary or femoral arteries. For each animal, necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination. The following organs were dissected free of fat and weights recorded: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, and thyroid/parathyroids. For each animal, approximately 40 protocol-selected tissues (and any gross lesions) were retained in neutral buffered 10% formalin, except for eyes, optic nerves, epididymides, and testes that were retained in Zenker's fixative. All tissues from all main study and recovery animals from all groups were prepared for histopathological examination by embedding in paraffin wax, sectioning, and staining with hematoxylin and eosin. Histopathological examination was performed on all retained tissues from all main study and recovery animals. Femoral bone marrow smears were also prepared for each euthanized animal, stained with May–Grünwald–Giemsa, and evaluated quantitatively to calculate myeloid:erythroid cell ratios.

Toxicokinetic analysis. Blood samples (approximately 1 ml each) were drawn from all main study animals (7 samples/animal/sampling day) for toxicokinetic analysis predose and 5 min, 30 min, 1, 2, 4, and 6 h postdose on Day 1, and during Weeks 13, 26, 39, and 52. Blood was collected into tubes containing sodium heparin. Plasma samples were placed on ice and stored at circa −20°C until analyzed. The concentrations of cidofovir in monkey plasma were determined at Genzyme Mason Laboratories, Inc. (Worcester, MA) under GLP conditions using a validated reversed-phase ion-pair HPLC method modified slightly from the published human serum method (Cundy *et al.*, 1995). Pharmacokinetic parameters for cidofovir were assessed using noncompartmental methods by application of PCNONLIN.

Analysis of data. Means and standard deviations were analyzed for body weights, food consumption, absolute and relative organ weights, and clinical chemistry and hematology parameters. Data from test groups were analyzed for homogeneity of variance using Bartlett's test. Homogeneous data were analyzed by ANOVA and the significance of intergroup differences was assessed using Dunnett's t test. Heterogeneous data were analyzed using the Kruskal–Wallis test and the significance of intergroup differences was assessed using Dunn's test. The effect of dose, sex, and probenecid cotreatment on pharmacokinetic parameters of cidofovir was assessed by the unpaired nonparametric test (Mann–Whitney U test) using the software package STATVIEW. The dose proportionality of C_{max} and AUC ($0 \rightarrow \infty$) was assessed using an unpaired t test to compare dose-normalized values.

RESULTS

52-Week Treatment Cohorts

In-life findings. The major findings of chronic toxicity associated with cidofovir treatment in cynomolgus monkeys are summarized in Table 1. In the 52-week treatment cohort, no unscheduled deaths occurred and no test article-associated effects were observed as measured by overall clinical signs of toxicity or changes in ophthalmological or food consumption parameters. Terminal body weights were significantly decreased in males receiving 2.5 mg/kg/dose cidofovir alone or in combination with orally administered probenecid (67 and 78% of control group values, respectively; Table 2).

TABLE 2
Group Mean Terminal Body Weight and Organ Weight Values[a]

Group	Terminal body weight (kg)	Liver		Kidney		Spleen		Testes	
		Absolute (g)	Relative (g/kg)	Absolute (g)	Relative (g/kg)	Absolute (g)	Relative (g/kg)	Absolute (g)	Relative (g/kg)
					Males				
1	5.2 (0.8)	85.0 (10.8)	16.3 (0.7)	18.8 (1.6)	3.6 (0.3)	9.4 (1.7)	1.8 (0.3)	16.4 (4.2)	3.2 (0.9)
2	4.6 (0.8)	82.6 (11.0)	17.9 (1.0)	17.3 (2.1)	3.8 (0.4)	7.6 (1.1)	1.7 (0.2)	13.4 (7.6)	2.9 (1.9)
3	4.4 (0.7)	75.1 (13.6)	17.0 (1.3)	16.9 (2.2)	3.9 (0.3)	9.9 (1.7)	2.2 (0.2)	12.1 (8.9)	2.6 (1.8)
4	3.5 (0.4)[b]	75.2 (14.3)	21.6 (4.4)[b]	17.5 (4.3)	5.0 (0.9)[b]	6.7 (0.8)[c]	1.9 (0.2)	5.6 (2.5)[c]	1.6 (0.7)
5	4.1 (0.3)[c]	74.8 (6.1)	18.2 (1.7)	15.6 (1.0)	3.8 (0.1)	8.7 (1.9)	2.1 (0.5)	5.3 (2.5)[c]	1.3 (0.6)
					Females				
1	3.1 (0.3)	60.1 (8.1)	19.1 (1.6)	13.3 (1.4)	4.3 (0.4)	6.2 (1.0)	2.0 (0.3)	0.40 (.10)	0.13 (0.03)
2	3.3 (0.3)	60.5 (5.0)	18.6 (2.1)	12.8 (1.2)	4.0 (0.7)	7.1 (2.4)	2.1 (0.6)	0.44 (.10)	0.14 (0.03)
3	3.4 (0.5)	65.5 (6.9)	19.4 (1.7)	13.0 (0.9)	3.9 (0.6)	6.9 (2.6)	2.0 (0.6)	0.43 (.10)	0.13 (0.03)
4	2.8 (0.2)	54.7 (2.4)	19.6 (0.9)	15.0 (1.8)	5.4 (0.8)[c]	5.2 (1.1)	1.8 (0.4)	0.31 (.13)	0.11 (0.05)
5	3.3 (0.4)	65.9 (10.1)	20.1 (1.2)	13.4 (1.7)	4.1 (0.5)	6.7 (1.4)	2.0 (0.3)	0.41 (.10)	0.13 (0.04)

[a] Cidofovir was administered as a single intravenous bolus injection once weekly for 52 consecutive weeks to cynomolgus monkeys (7/sex/Groups 1, 4, and 5; 5/sex/Groups 2 and 3) at 0, 0.1, 0.5, or 2.5 mg/kg/day alone (Groups 1–4) or at 2.5 mg/kg/dose in combination with orally administered probenecid (30 mg/kg 1 h predose, Group 5). Data reflect group mean (±SD) values for terminal body weights (kg) or organ weights [absolute (g) or relative to body weight (g/kg)].
[b,c] Statistical significance: [b] $p < 0.01$, [c] $p < 0.05$ (Dunnett's test).

Clinical pathology. Effects on blood biochemistry and urinalysis parameters were observed only in the group receiving cidofovir at 2.5 mg/kg/dose without probenecid. Significant increases in creatinine levels occurred starting from Week 25 until treatment termination in males (188 to 277% of control values) and females (122 to 144% of control values; Table 3). Blood urea nitrogen also increased in males (173 to 276% of control values) from Week 25 to treatment termination. Moderate nonsignificant increases in alkaline phosphatase were present in terminal blood samples in males and females (209 and 160% of respective control group values; data not shown). Creatinine, blood urea nitrogen, and alkaline phosphatase values were elevated in one of two recovery group males administered cidofovir at 2.5 mg/kg/dose alone for 52 weeks followed by a 4-week nontreatment period. At treatment termination, males and females administered 2.5 mg/kg/dose cidofovir without probenecid showed evidence of glucosuria and proteinuria, but no changes in urine pH or volume (Table 4). No cidofovir-associated changes in hematological parameters occurred in any treatment group.

Organ weights. Organ weight changes attributable to cidofovir treatment occurred only in the high-dose cohorts. Absolute testes weights were reduced significantly in groups that received 2.5 mg/kg/dose cidofovir alone or with coadministered probenecid (67 and 69% of control values, respectively; Table 2). Relative testes weights were also reduced nonsignificantly in these groups. In the high-dose group administered cidofovir alone, relative kidney weights were higher in males and females, whereas absolute spleen weights were decreased and relative liver weights were increased in males only. Other organ weights were unchanged.

Microscopic findings. Histopathological changes associated with chronic cidofovir administration were seen in the kidneys, testes, and epididymides. Present only in the group receiving 2.5 mg/kg/dose cidofovir without probenecid was a renal lesion that was characterized by a mild to moderate cortical tubular epithelial cell karyomegaly, tubular dilatation, basement membrane thickening, and increased mononuclear cell infiltration (Fig. 1). No evidence of tubular epithelial cell degeneration or necrosis or renal interstitial fibrosis was detected. In this group, nephrotoxic effects were present in all main study and recovery animals and were of comparable severity, suggesting no sex-related differences in sensitivity and no apparent lesion reversibility following the 4-week nontreatment period.

Testicular changes characterized by mild hypo- and aspermatogenesis were present in monkeys in the control (1 of 5), low-dose (3 of 5), and mid-dose (3 of 5) treatment groups. This finding is common in young adult cynomolgus monkeys, as used in this study, but varies in severity with animal age and sexual maturity. However, based on both the higher incidence (5 of 5 males) and severity (moderate to severe) of these testicular effects and the lower absolute and relative testes weights in both of the high-dose groups (with and without probenecid), a test article-related effect cannot be excluded. Testicular histopathological changes were limited to spermatogenic cells; no effects were observed in Leydig or Sertoli cells. The moderate to severe epididymal oligo- and aspermia

TABLE 3
Group Mean Blood Urea Nitrogen (BUN) and Creatinine Values[a]

Group	Pretreatment	Week 25	Week 38	Week 51	Week 56
			BUN (mg/dl)—Males		
1	18.0 (1.1)	17.9 (2.0)	20.0 (3.4)	16.0 (1.3)	17.9 (1.7)
2	22.3 (3.2)	22.4 (3.6)	23.8 (4.8)	20.9 (4.9)	NS
3	20.9 (2.9)	21.2 (1.2)	22.6 (3.4)	19.1 (1.1)	NS
4	18.6 (2.6)	31.1 (25.6)	36.3 (39.7)	44.2 (52.7)	35.7 (4.0)
5	19.9 (2.6)	19.3 (2.4)	19.2 (2.2)	19.1 (3.1)	21.0 (1.1)
			BUN (mg/dl)—Females		
1	19.2 (4.4)	18.1 (3.3)	19.1 (2.6)	17.8 (3.2)	20.3 (2.0)
2	19.9 (2.7)	20.4 (2.5)	19.0 (2.6)	20.5 (3.6)	NS
3	17.3 (1.4)	19.1 (1.5)	20.5 (3.6)	18.9 (1.9)	NS
4	19.0 (2.4)	18.8 (5.0)	20.1 (4.8)	23.7 (6.1)	21.7 (7.28)
5	20.9 (2.6)	19.6 (3.0)	20.8 (3.1)	18.3 (4.0)	28.4 (2.2)
			Creatinine (mg/dl)—Males		
1	0.8 (0.2)	0.9 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)
2	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)	NS
3	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)	NS
4	0.8 (0.2)	1.7 (1.1)[b]	1.8 (1.6)	2.5 (2.2)[c]	2.5 (0.7)
5	0.8 (0.1)	1.0 (0.1)	0.9 (0.1)	1.0 (0.1)	1.0 (0)
			Creatinine (mg/dl)—Females		
1	0.9 (0.2)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0)
2	0.9 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.1)	NS
3	0.9 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.1)	NS
4	0.8 (0.1)	1.1 (0.1)[c]	1.2 (0.2)[d]	1.3 (0.2)[d]	1.3 (0.4)
5	0.9 (0.2)	1.1 (0.2)	0.9 (0.2)	0.9 (0.1)	0.9 (0)

[a] Cidofovir was administered as a single intravenous bolus injection once weekly for 52 consecutive weeks to cynomolgus monkeys at 0, 0.1, 0.5, or 2.5 mg/kg/day alone (Groups 1–4), at 2.5 mg/kg/dose, or in combination with orally administered probenecid (30 mg/kg 1 h predose, Group 5). Data reflect group mean (±SD) values from blood samples taken pretreatment through treatment termination (7/sex/Groups 1, 4, and 5; 5/sex/Groups 2 and 3) or following a 4-week nontreatment period (2/sex/Groups 1, 4, and 5).

[b–c] Statistical significance: [b] $p < 0.05$ (Dunn's); [c] $p < 0.05$ (Dunnett's); [d] $p < 0.01$ (Dunn's); [e] $p < 0.01$ (Dunnett's). NS, no sample.

also present in all high-dose males was considered a change secondary to the testicular effects. Although the testicular and epididymal effects in recovery control and high dose cohorts were of comparable incidence and severity, the reversibility of these lesions following nontreatment for 4 weeks could not be ascertained due to the small group size (2 male animals).

26-Week Treatment Cohorts

In the five males and five females receiving a 2.5 mg/kg/dose cidofovir in combination with probenecid for 26 consecutive weeks, no unscheduled deaths occurred and no test article-associated effects were observed as measured by clinical signs of toxicity or changes in clinical chemistry, urinalysis, hematology, ophthalmoscopy, food consumption, body weights, organ weights, gross necropsy findings, or histomorphological appearance. No treatment cohort received cidofovir alone for only 26 weeks.

Toxicokinetic Analysis

Initial concentrations of cidofovir in plasma (C_0) and cidofovir AUC values at Week 1 were dose proportional over the cidofovir dose range of 0.1 to 2.5 mg/kg/dose in the absence of probenecid (Table 5). However, both C_0 and AUC values showed significant increases by Week 52 in monkeys administered cidofovir at 2.5 mg/kg/dose without probenecid ($p = 0.02$ and $p < 0.01$, respectively). The magnitude of the increase in mean AUC from Week 1 was greater in male monkeys (312%) than in female monkeys (98%). Terminal half-life was increased over the treatment period only in males receiving cidofovir alone at 2.5 mg/kg/dose.

A probenecid-mediated block of the cidofovir tubular secretion pathway was indicated based on the greater initial (Week 1) AUC values in males (24%) and females (86%) coadministered oral probenecid compared with monkeys that received intravenous cidofovir alone at 2.5 mg/kg/dose. Over the 52-week treatment period, probenecid coadministration resulted in smaller decreases in the volume of distribution and clearance of cidofovir in both males and females and in smaller increases in AUC values (32% in males and 3% in females; Fig. 2).

DISCUSSION

The present study is the first nonclinical chronic toxicity evaluation of a new class of antiviral drugs (nucleotide analogues) that are currently in clinical trials for a variety of human diseases, including HIV and hepatitis B infections. Cidofovir is the first nucleotide analogue ever approved for clinical use (CMV retinitis in HIV-infected patients). The toxicologic and pharmacokinetic data from this study show an unequivocal nephroprotective effect for a clinically relevant oral probenecid dose regimen that is currently used with intravenous cidofovir therapy. The importance of these data lies in the inability to do (for ethical reasons) a controlled clinical trial in the presence and absence of probenecid and illustrates the proper utility of animal models in the drug development process.

Cidofovir administered intravenously to cynomolgus monkeys once weekly for 52 weeks produced no significant adverse effects at 0.1 or 0.5 mg/kg/week. However, at 2.5 mg/kg/week dose, cidofovir treatment resulted in histopathological changes in kidneys, testes, and epididymides. The light microscopic findings in kidney tissue (i.e., cortical tubular epithelial cell karyomegaly, tubular dilatation, basement membrane thickening, and increased mononuclear cell infiltration) and associated findings indicative of nephrotoxicity (i.e., increased BUN and creatinine, glucosuria, and proteinuria) present at the 2.5 mg/

TABLE 4
Group Mean Urinalysis Values[a]

Group	Protein (g/liter)		Glucose (mmol/liter)		pH		Volume (ml)	
	Pretreatment	Week 50	Pretreatment	Week 50	Pretreatment	Week 50	Pretreatment	Week 50
				Males				
1	ND	ND	ND	ND	7.9 (0.9)	7.8 (1.3)	70.1 (32.8)	129.9 (107)
2	0.1 (0.1)	ND	ND	ND	7.2 (2.1)	7.5 (0.9)	37.6 (20.7)	87.8 (26.3)
3	0.2 (0.4)	ND	ND	ND	6.9 (1.0)	7.4 (1.3)	36.6 (27.1)	84.0 (23.5)
4	0.1 (0.1)	0.3 (0.4)	ND	11 (12)	7.1 (0.8)	6.9 (1.1)	33.1 (18.1)	138.1 (92.8)
5	ND	ND	ND	ND	7.7 (1.0)	7.5 (1.0)	40.3 (21.3)	103.4 (49.7)
				Females				
1	0.1 (0.1)	ND	ND	ND	8.5 (0.9)	8.1 (1.1)	37.9 (22.3)	77.7 (38.1)
2	ND	ND	ND	ND	8.4 (0.4)	7.3 (0.6)	46.7 (16.8)	45.4 (29.5)
3	0.1 (0.2)	ND	ND	ND	8.0 (1.2)	8.2 (0.9)	38.0 (15.1)	58.2 (17.8)
4	ND	0.4 (0.3)	ND	25.7 (23)	7.8 (1.7)	7.9 (0.8)	34.8 (8.4)	73.7 (19.5)
5	0 (0.1)	ND	ND	ND	7.9 (1.1)	7.1 (1.3)	38.6 (15.9)	51.0 (35.6)

[a] Cidofovir was administered as a single intravenous bolus injection once weekly for 52 consecutive weeks to cynomolgus monkeys (7/sex/Groups 1, 4, and 5; 5/sex/Groups 2 and 3) at 0, 0.1, 0.5, or 2.5 mg/kg/day alone (Groups 1–4) or at 2.5 mg/kg/dose in combination with orally administered probenecid (30 mg/kg 1 h predose, Group 5). Data reflect group mean (±SD) values. ND, not detected.

kg/week cidofovir dose were not detected in monkeys receiving concomitant oral probenecid treatment. Oral probenecid administration also protected against the decrease in renal clearance of cidofovir that occurred over time at the 2.5 mg/kg/week dose. However, the testicular and epididymal lesions observed at the 2.5 mg/kg/week cidofovir dose were unaffected by probenecid cotreatment. These data demonstrate that oral probenecid coadministration provided an effective nephroprotective effect in cynomolgus monkeys receiving chronic intravenous cidofovir treatment.

Nephrotoxicity

The reduced cidofovir-related nephrotoxicity seen in cynomolgus monkeys administered probenecid is believed to result from decreased tubular epithelial cell uptake and lower kidney tissue drug levels relative to monkeys given cidofovir alone. A tubular-transport mechanism of nephrotoxicity has been proposed previously for several structurally dissimilar chemicals, including the β-lactam antibiotic cephaloridine, the cysteine conjugate of trichloroethylene DCVC, and the fungal toxin citrinin (for reviews see Berndt, 1989; Tune, 1993). Nephrotoxic β-lactams, including cephaloridine, produce a selective necrosis of the proximal renal tubular epithelial cells. Cephaloridine is a zwitterion and is rapidly taken up into the proximal renal tubular epithelial cell by a probenecid-sensitive transporter on the antiluminal (blood) side. However, movement of this cephalosporin across the luminal surface into the tubular fluid is greatly restricted, presumably related to its cationic charge. Intracellular trapping of cephaloridine can lead to renal cortical tissue drug levels in rabbits more than 15-fold greater than respective serum concentrations. In addition, the species-sensitive nephrotoxicity of cephaloridine correlates with differences in the extent of accumulation of drug in kidney tissue. Relative tubular epithelial cell uptake is also believed to underlie the nephrotoxic potential of other charged β-lactams (e.g., cephaloglycin), whereas the lower nephrotoxic potential of uncharged cephalosporins (e.g., cefaclor) is thought to be a consequence of their more efficient tubular epithelial cell secretion and lower intracellular sequestration.

The renal toxicity associated with the nucleotide analogue cidofovir is consistent with the tubular-transport mechanism proposed for nephrotoxic β-lactam antimicrobials. Cidofovir is a dianion at physiological pH; its uptake into the proximal tubular epithelial cell appears to be mediated by a probenecid-sensitive organic anion transporter. However, once inside the tubule cell, cidofovir's anionic charge may be responsible for a slow diffusion rate across the luminal membrane leading to the high renal cortical tissue drug levels observed previously in rabbits and monkeys (Cundy *et al.*, 1994, 1996a, b). This premise is supported by preclinical studies of cyclic 1-[(*S*)-3-hydroxy-2-phosphonomethoxy)propyl]cytosine (cyclic HPMPC), an analogue of cidofovir with a single negative charge. Compared with cidofovir, cyclic HPMPC is 10- to 40-fold less nephrotoxic (Bischofberger *et al.*, 1994), concentrates approximately 20-fold less in rat kidney tissue (Cundy *et al.*, 1996c), and displays more efficient renal tubular epithelial cell secretion. The reduced charge on cyclic HPMPC results in enhanced active transport across the luminal surface, thereby decreasing tissue drug accumulation and nephrotoxic potential relative to cidofovir.

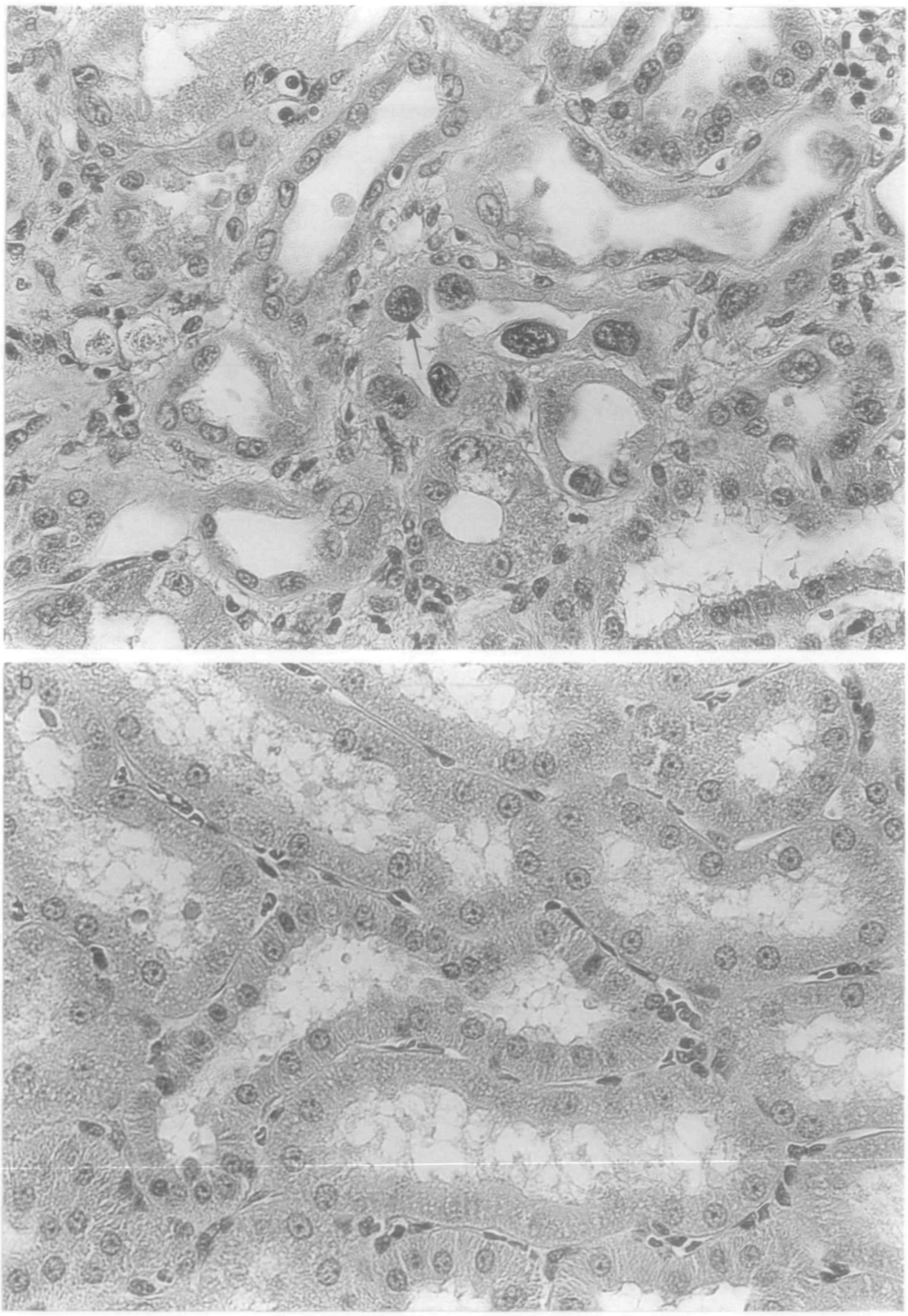

FIG. 1. Kidney cortex from male cynomolgus monkeys administered cidofovir intravenously once weekly for 52 consecutive weeks at 2.5 mg/kg/dose: (a) tubular epithelial cell karyomegaly (arrow), tubular dilation, and basement membrane thickening in animals given cidofovir alone; (b) normal renal appearance in animals receiving concomitant probenecid (30 mg/kg po 1 h predose). Hematoxylin and eosin stain; ×440.

Effect of Probenecid

The nephroprotective effect of probenecid, observed with cidofovir in the present study, has been demonstrated previously for drug substrates of the renal organic anion transport system, including citrinin in rats (Berndt, 1983) and cephalosporin in several animal models (Tune and Flavert, 1980a, b). Prevention of cephalosporin renal toxicity required probenecid doses sufficient to inhibit active uptake of drug at the antiluminal surface of the proximal tubular epithelial cell until circulating antibiotic levels declined substantially. The probenecid dosage administered to cidofovir-treated cynomolgus monkeys (30 mg/kg po 1 h prior to cidofovir) was based on achieving a dose comparable (on a mg/kg basis) to the initial dose (2 g po 3 h prior to iv cidofovir 1-h infusion) being used clinically. In humans, probenecid absorption following oral administration is reported to be rapid and complete, with peak plasma concentrations occurring in 1 to 5 h and a plasma half-life ranging from 4 to 12 h (Cunningham *et al.*, 1981). In contrast, the plasma half-life for intravenous cidofovir determined in the present study for monkeys (range, 0.5–3.4 h) is much shorter. In a previous study (Cundy *et al.*, 1996a), the pharmacokinetics of intravenous [^{14}C]cidofovir in African Green monkeys was shown to fit a three-compartment model (α, β and γ plasma half-lives of 0.67, 3.02, and 36 h, respectively). The oral probenecid dosage used in the present study completely prevented appearance of the renal histopathological changes in cynomolgus monkeys administered intravenous cidofovir at 2.5 mg/kg/week for 52 weeks. In contrast, in an earlier 13-week study in cynomolgus monkeys, this oral probenecid dosage regimen only partially protected against histological kidney lesions when cidofovir was administered at a twofold larger intravenous dosage (5 mg/kg/week) (Lacy and Hitchcock, unpublished data).

TABLE 5

Pharmacokinetic Parameters for Cidofovir Administered Intravenously Once Weekly for 52 Consecutive Weeks to Male and Female Cynomolgus Monkeys at 0.1, 0.5, or 2.5 mg/kg/Dose Alone or at 2.5 mg/kg/dose in Combination with Orally Administered Probenecid (30 mg/kg 1 h Predose)[a]

Dose	Sex	Week	C_0 (μg/ml)	AUC (0–∞) (μg · h/ml)	$t_{\frac{1}{2}}$ (h)	CL (ml/h/kg)	V_{ss} (ml/kg)
0.1	M	1	0.55	0.24	0.46	437	294
		52	0.52	0.29	0.45	351	229
	F	1	0.73	0.74	2.50	144	496
		52	0.60	0.35	0.52	286	213
0.5	M	1	2.64	1.69	1.07	307	470
		52	3.28	2.02	0.90	248	322
	F	1	3.46	2.06	1.14	249	414
		52	2.71	2.06	1.17	243	409
2.5	M	1	14.7	8.77	1.04	299	444
		52	18.3	36.1	2.69	142	297
	F	1	13.8	8.48	1.23	301	532
		52	17.4	16.8	1.20	160	273
2.5 + probenecid	M	1	14.1	10.9	1.00	240	350
		52	17.9	14.4	0.94	174	234
	F	1	21.9	15.8	1.01	163	235
		52	19.6	16.3	0.91	153	201

[a] Values are group means (5/sex/group). C_0, initial concentration of cidofovir in plasma; AUC (0–∞), area under the plasma concentration-versus-time curve extrapolated to infinity; $t_{\frac{1}{2}}$, terminal-phase high-life; CL, cidofovir clearance; V_{ss}, steady-state volume of distribution.

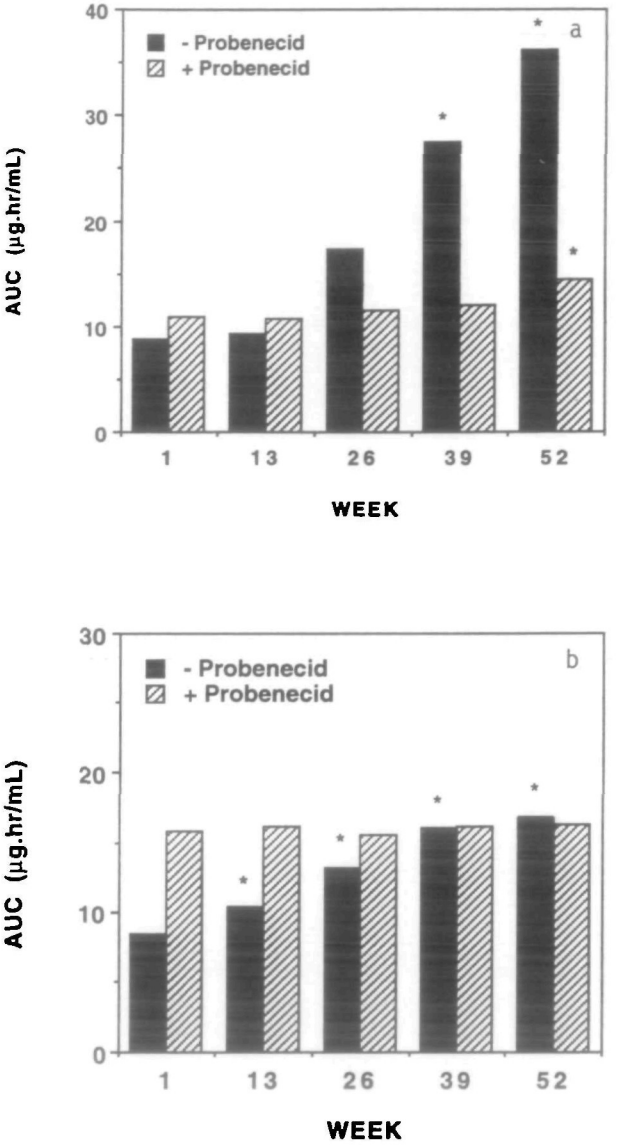

FIG. 2. Mean pharmacokinetic area-under-the curve (AUC) values for cidofovir in plasma samples from (a) male and (b) female cynomolgus monkeys administered cidofovir intravenously once weekly for up to 52 consecutive weeks at 2.5 mg/kg/dose alone (solid bar) or in combination with orally administered probenecid (30 mg/kg 1 h predose; striped bar). *Significantly different from Day 1 ($p < 0.05$).

Pharmacokinetic Changes

Probenecid coadministration altered cidofovir-mediated effects on renal drug clearance. In the absence of probenecid, plasma clearance rates in male and female cynomolgus monkeys administered 2.5 mg/kg/dose cidofovir decreased progressively over the course of the study to approximately 50% of initial levels by treatment termination. Corresponding increases in plasma AUC levels in males and females of 312 and 98%, respectively, occurred over this period. Decreased cidofovir clearance associated with kidney toxicity has been reported previously in African Green monkeys not receiving concomitant probenecid (Cundy *et al.,* 1996a). In the present study, concomitant oral probenecid treatment resulted in smaller decreases in plasma cidofovir clearance (28% in males, 7% in females) and consequently smaller increases in AUC (32% in males, 3% in females). The magnitude of the change in cidofovir clearance produced by repeated dosing was greater in males than in females. This suggests that male monkeys possess a higher capacity for renal anion transport than females. Similar sex differences have been previously demonstrated for an organic anion transport system (OATP) in rats, the expression of which is under hormonal control (Lu *et al.,* 1996). However, in the present study, no sex-related differences were observed in kidney lesion severity, indicating that the degree of histopathological change did not correlate directly with effects on cidofovir clearance. In clinical trials, probenecid coadminstration was shown to protect against evidence of cidofovir-induced nephrotoxicity (proteinuria) and to reduce renal drug clearance to a level approximating glomerular filtration, presumably by blocking the tubular secretion pathway (Cundy *et al.,* 1995).

SUMMARY

The chronic toxicity of intravenous cidofovir in cynomolgus monkeys was associated with histomorphological changes in kidney, testes, and epididymides at the 2.5 mg/kg/week dose. Coadministration of a clinically relevant oral probenecid dosage completely prevented cidofovir-mediated nephrotoxicity based on absence of light microscopic changes in the kidney tubular epithelial cells and associated nephrotoxic effects on clinical biochemistry parameters (i.e., increased blood urea nitrogen and creatinine, glucosuria, proteinuria). Concomitant probenecid treatment provided significant protection against the decrease in renal cidofovir clearance. The lack of probenecid protection of the cidofovir-associated testicular or epididymal lesions strongly supports the assumption of a selective inhibition of the organic anion transporter of the proximal tubular epithelium leading to decreased cellular uptake and lower intracellular levels of cidofovir. The incomplete reversibility in testicular and kidney lesions following 4 nontreatment weeks may reflect the long intracellular half-lifes of cidofovir metabolites, the slow turnover of the target cell populations, and/or the short recovery period. Results from this study extend our knowledge regarding the effect of probenecid on cidofovir nephrotoxicity in a chronic study of 12-month duration. Moreover, these data confirm clinical findings of the significantly enhanced safety profile provided by oral probenecid for treatment of CMV retinitis by cidofovir injection.

ACKNOWLEDGMENT

We thank Debra Farrell at Clintrials BioResearch Laboratories Ltd (Senneville, Quebec) for her contributions on the in-life portion of this study.

REFERENCES

Berndt, W. O. (1983). Transport of citrinin by rat renal cortex. *Arch. Toxicol.* **54,** 35–40.

Berndt, W. O. (1989). Potential involvement of renal transport mechanisms in nephrotoxicity. *Toxicol. Lett.* **46,** 77–82.

Bischofberger, N., Hitchcock, M. J. M., Chen, M. S., Cundy, K. C., Barkhimer, D., Kent, K. M., Lacy, S. A., Lee, W. A., Li, Z.-H., Louie, M., Mendel, D. B., Smee, D. S., and Smith, J. L. (1994). Cyclic HPMPC: An intracellular prodrug for HPMPC with improved therapeutic index *in vivo. Antimicrob. Agents Chemother.* **38,** 2387–2391.

Cihlar, T., Votruba, I., Horska, K., Liboska, R., Rosenberg, I., and Holy, A. (1992). Metabolism of 1- (*S*)-(3-hydroxy-2-phosphonomethoxy propyl)-cytosine (HPMPC) in human embryonic lung cells. *Collect. Czech. Chem. Commun.* **57,** 661–672.

Cundy, K. C., Li, Z.-H., and Lee, W. A. (1994). Effect of concomitant probenecid on the tissue distribution and urinary excretion of HPMPC in preclinical models. *Pharm. Res.* **11**(Suppl.), S449.

Cundy, K. C., Petty, B. G., Flaherty, J., Fisher, P. E., Polis, M. A., Wachsman, M., Lietman, P. S., Lalezari, J. P., Hitchcock, M. J. M., and Jaffe, H. S. (1995). Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* **39,** 1247–1252.

Cundy, K. C., Li, Z.-H., Hitchcock, M. J. M., and Lee, W. A. (1996a). Pharmacokinetics of cidofovir in monkeys. Evidence for a prolonged elimination phase representing phosphorylated drug. *Drug Metab. Dispos.* **24,** 738–744.

Cundy, K. C., Li, Z.-H., and Lee, W. A. (1996b). Effect of probenecid on the distribution, metabolism, and excretion of cidofovir in rabbits. *Drug Metab. Dispos.* **24,** 315–321.

Cundy, K. C., Bidgood, A., M., Lynch, G., Shaw, J.-P., Griffin, L., and Lee, W. A. (1996c). Pharmacokinetics, bioavailability, metabolism, and tissue distribution of cidofovir (HPMPC) and cyclic HPMPC in rats. *Drug Metab. Dispos.* **24,** 745–752.

Cunningham, R. F., Israili, Z. H., and Dayton, P. G. (1981). Clinical pharmacokinetics of probenecid. *Clin. Pharmacokinet.* **6,** 135–151.

Hitchcock, M. J. M., Jaffe, H. S., Martin, J. C. and Stagg, R. J. (1996). Cidofovir, a new agent with potent anti-herpes activity. *Antiviral Chem. Chemother.* **7,** 115–127.

Ho, H.-T., Woods, K. L., Bronson, J. J., De Boeck, H., Martin, J. C., and Hitchcock, M. J. M. (1992). Intracellular metabolism of the antiherpes agent (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine. *Mol. Pharmacol.* **41,** 197–202.

Lalezari, J. P., Drew, W. L., Glutzer, E., James, C., Miner, D., Flaherty, J., Fisher, P. E., Cundy, K., Hannigan, J., Martin, J. C., and Jaffe, H. S. (1995). (*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl] cytosine (Cidofovir): Results of a phase I/II study of a novel antiviral nucleotide analogue. *J. Infect. Dis.* **171,** 788–796.

Lalezari, J. P., Stagg, R. J., Kupperman, B. D., Holland, G. N., Kramer, F., Ives, D. V., Youle, M., Robinson, M. R., Drew, W. L., and Jaffe, H. S. (1997). Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS: A randomized, controlled trial. *Ann. Intern. Med.* **126,** 257–263.

Lu, R., Kanai, N., Bao, Y., Wolkoff, A. W., and Schuster, V. L. (1996). Regulation of renal oatp mRNA expression by testosterone. *Am. J. Physiol.* **270,** F332–337.

Naesens, L., Snoeck, R., Andrei, G., Balzarini, J., Neyts, J., and De Clercq, E. (1997). HPMPC (cidofovir), PMEA (adefovir), and related acyclic nucleoside phosphonate analogues: A review of their pharmacology and clinical potential in the treatment of viral infections. *Antiviral Chem. Chemother.* **8,** 1–23.

Studies of Ocular Complication of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group (SOCA) (1997). Parenteral cidofovir for cytomegalovirus retinitis in patients with AIDS: the HPMPC peripheral cytomegalovirus retinitis trial. *Ann. Intern. Med.* **126,** 264–274.

Tune, B. N. (1993). The nephrotoxicity of β-lactam antibiotics. In *Toxicology of the Kidney* (J. B. Hook and R. S. Goldstein, Eds.), 2nd ed., pp. 257–281. Raven Press, New York.

Tune, B. M., and Flavert, D. (1980a). Cephalosporin nephrotoxicity: Transport, cytotoxicity and mitochondrial toxicity of cephaloglycin. *J. Pharmacol. Exp. Ther.* **215,** 186–190.

Tune, B. M., and Flavert, D. (1980b). Mechanisms of cephalosporin nephrotoxicity: A comparison of cephaloridine and cephaloglycin. *Kidney Int.* **18,** 591–600.

Vlasses, P. H., Holbrook, A. M., Schrogie, J. J., Rogers, J. D., Ferguson, R. K., and Abrams, W. B. (1980). Effect of orally administered probenecid on the pharmacokinetics of cefoxitin. *Antimicrob. Agents Chemother.* **17,** 847–855.