Phase I Dose Escalation Trial Evaluating the Pharmacokinetics, Anti-Human Cytomegalovirus (HCMV) Activity, and Safety of 1263W94 in Human Immunodeficiency Virus-Infected Men with Asymptomatic HCMV Shedding

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Infection with human cytomegalovirus (HCMV) is common, with seroprevalence ranging from approximately 50 to 60% of adults in Western Europe and the United States to as much as 100% of some adult populations (6, 13). In immunocompetent HCMV-infected individuals, the virus normally remains latent and does not constitute a major health risk. However, HCMV infection can be a serious complication in immunologically immature individuals, such as neonates, or in immunocompromised individuals, such as solid-organ transplant recipients, bone marrow transplant recipients, or people with AIDS.

There are six currently approved therapies in the United States for treatment or prevention of systemic HCMV infection or HCMV retinitis associated with AIDS (6, 13). Treatment of systemic HCMV infection requires administration of ganciclovir, foscarnet, or cidofovir. After induction therapy with intravenously (i.v.) administered agents or oral valganciclovir, maintenance therapy can be provided by oral ganciclovir together with intravenously (i.v.) administered agents or oral valganciclovir. Fomivirsen, administered by intravitreal injection, is approved as a second-line therapy for treatment of HCMV retinitis in subjects who cannot tolerate or fail to respond to first-line therapy. Disadvantages of the currently approved therapies include treatment-limiting toxicities, such as bone marrow suppression (ganciclovir) and nephrotoxicity (foscarnet and cidofovir), limited penetration to target sites, and inconvenient i.v. dosing or poor oral bioavailability (oral ganciclovir) (13). Thus, there is a need for a safe and effective oral therapy for the treatment and prevention of HCMV disease.

1263W94 [maribavir; 5,6-dichloro-2-(isopropylamino)-1,β-L-ribofuranosyl-1-H-benzimidazole] is a novel benzimidazole compound for treatment of human cytomegalovirus (HCMV) infection and disease, with potent in vitro activity against HCMV and good oral bioavailability. A phase I study was conducted to determine the pharmacokinetics (PK), anti-HCMV activity, and safety of 1263W94 administered as multiple oral doses to human immunodeficiency virus type 1-infected adult male subjects with asymptomatic HCMV shedding. Subjects received one of six dosage regimens (100, 200, or 400 mg three times a day, or 600, 900, or 1,200 mg twice a day) or a placebo for 28 days. 1263W94 demonstrated linear PK, with steady-state plasma 1263W94 profiles predictable based on single-dose data. 1263W94 was rapidly absorbed following oral dosing, and values for the maximum concentration of the drug in plasma and the area under the concentration-time curve increased in proportion to the dose. 1263W94 demonstrated in vivo anti-HCMV activity in semen at all of the dosage regimens tested, with mean reductions in semen HCMV titers of 2.9 to 3.7 log10 PFU/ml among the four regimens evaluated for anti-HCMV activity. 1263W94 was generally well tolerated; taste disturbance was the most frequently reported adverse event over the 28-day dosing period.

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There was no requirement for detectable HCMV infection in subjects in the main group for evaluation of the PK, anti-HCMV activity, and safety of 1263W94 dose recovered in the urine was recovered as the N-dealkylated metabolite (Wang et al., unpublished).

We conducted a phase I dose escalation study to evaluate the PK, anti-HCMV activity, and safety of 1263W94 in HIV-1-infected adult males with asymptomatic HCMV shedding in urine and semen. Quantitative reductions in semen HCMV concentrations are dose responsive and predictive of clinical efficacy (10–12).

MATERIALS AND METHODS

Study population and investigative sites. The study population consisted of HIV-1-infected males. Eligibility criteria were as follows: all subjects had to be ≥18 years old, have a life expectancy of >6 months, and be stable on all chronically administered therapy for HIV infection and opportunistic infections for at least 1 month. Subjects were stratified into two groups, main and satellite, on the basis of semen and urine HCMV culture results prior to study entry. Subjects with a semen HCMV concentration of ≥5,000 PFU/ml and with an HCMV-positive urine culture within 30 days of study entry (day 1) were enrolled in the main group for evaluation of the PK, anti-HCMV activity, and safety of 1263W94. There was no requirement for detectable HCMV infection in subjects enrolled in the satellite group for evaluation of 1263W94 PK and safety. Subjects enrolled in the satellite group were required to have a CD4+ cell count of <150 cells/mm³ or <10% of total lymphocytes. For subjects with a CD4+ cell count of <100 cells/mm³ or with signs or symptoms of HCMV disease, an indirect fundoscopic examination was performed by an ophthalmologist to rule out HCMV retinitis. The following exclusion criteria applied to all subjects: active HCMV disease or history of HCMV disease; visual symptoms suggestive of HCMV disease; or history of HCMV disease; known history of lactose intolerance; diagnosis of chronic diseases that could compromise the safety or compliance of the subject; treatment with radiation therapy or systemic therapy for visceral malignancy within 2 months prior to study entry (day 1); treatment with interferons, immunomodulatory agents, or HCMV hyperimmune globulin within 1 month prior to study entry (day 1); active hepatitis, obstructive hepatobiliary disease, or cirrhosis; gastrointestinal disorders that could interfere with oral dosing or drug absorption or that might induce HCMV disease; known history of lactose intolerance; diagnosis of chronic diseases that could compromise the safety or compliance of the subject; treatment with radiation therapy or systemic therapy for visceral malignancy within 2 months prior to study entry (day 1), or anticipated need for such treatment during the study period; participation in an investigational trial or treatment with an investigational therapy within 2 months (anti-HCMV therapy) or 1 month (other therapy) prior to study entry (day 1); abnormal laboratory values within 14 days of study entry (day 1), notably, hemoglobin of <8.5 g/dl, a neutrophil count of <750 cells/mm³, a platelet count of ≤50,000 cells/mm³, AST, ALT, or alkaline phosphatase levels >4 times the upper limit of normal, total bilirubin of >2 mg/dl, or estimated creatinine clearance of <50 ml/min; or a debilitated condition resulting from HIV disease or associated illnesses or therapies such that the subject was considered unable to complete the study.

The study was conducted from 27 August 1996 through 9 July 1997 at three sites in San Francisco, Calif. Quest Clinical Research, University of California San Francisco (UCSF) Mount Zion Medical Center, and San Francisco General Hospital were the three participating centers. The study was approved by the Western Institutional Review Board (Olympia, Wash.) and by the UCSF Committee on Human Research (San Francisco, Calif.) and was conducted under Good Clinical Practices guidelines. All subjects provided written informed consent before any study procedures were performed.

The Virology Research Laboratory at the UCSF Mount Zion Medical Center performed the plaque assays and processed samples for shipment to Glaxo-Wellcome for measurement of 1263W94 concentrations by validated assays.

Bioanalysis of PK samples. Plasma, urine, and semen samples were analyzed for 1263W94 by use of separately validated high-performance liquid chromatography-mass spectrometry (MS)-MS methods following solid-phase extraction. For plasma, the validated calibration range was 50 to 6,000 ng/ml, the accuracy (expressed as percent bias) was ±5.1%, and the global precision (expressed as the coefficient of variation [CV]) was 14.1%. For urine, the validated calibration range was 50 to 50,000 ng/ml, the accuracy (percent bias) was ±9.4%, and the global precision (CV) was 10.0%. For semen, the validated calibration range was 50 to 7,500 ng/ml, the accuracy (percent bias) was ±10.5%, and the global precision (CV) was 10.2%.

Plasma PK analysis. PK analyses of plasma 1263W94 concentration-versus-time data obtained following single-dose administration on day 1 and following multiple dosing to steady state on day 28 were conducted by noncompartmental methods with the log-linear trapezoidal option in WinNonLin Professional version 1.5 (Pharsight Corporation, Mountain View, Calif.). Values were estimated for the maximum concentration of 1263W94 in plasma (Cmax), and Cmax at steady state (Cmax,ss), time to maximum concentration (Tmax) and Tmax at steady state (Tmax,ss), minimum concentration during a dosing interval at steady state.
and strain 2916r was used as a reference 1263W94-resistant strain. 2916r is a collection, Manassas, Va.) was used as the wild-type HCMV reference strain, HCMV culture, 10- to 20-ml aliquots from each urine collection were refrigerated at °70 °C for 30 s for 30 cycles; and a hold at 72 °C for no more than 2 h. Quantitative detection of the PCR products was based on colorimetric detection of an avidin-horseradish peroxidase complex bound to the biotin moiety of the HCMV DNA primer.

**Results**

**Subject population.** Seventy-eight subjects were enrolled in the study. Of these, 28 were assigned to the main group and 50 were assigned to the satellite group. The number of subjects included in each of the 1263W94 dose cohorts is shown in Table 1. The 62 subjects receiving 1263W94 and the 16 subjects receiving the placebo were similar in demographic characteristics, in CD4 cell count, and in Centers for Disease Control and Prevention (CDC) classification status at baseline, as shown in Table 1.

Of the 78 subjects enrolled in the study, 70 completed the 28-day dosing period of the study. Eight subjects withdrew from the study prematurely. Of these eight, six subjects (three in the 200-mg t.i.d. cohort and one each in the 600-, 900-, and 1,200-mg b.i.d. cohorts) discontinued due to adverse events, consisting of grade-2 rash (five subjects) and sinusitis (one subject). A seventh subject (in the 900-mg b.i.d. cohort) was withdrawn from the study after the day-1 hematology results indicated neutropenia (400 cells/mm³), and an eighth subject (in the 900-mg b.i.d. cohort) withdrew from the study on day 2 due to anxiety about the serial PK sampling.

**Concomitant medications.** Subjects were stable on all chronically administered therapy for HIV and opportunistic infections for at least 1 month prior to receiving 1263W94. Most of the subjects were receiving concomitant medications that were CYP3A4 inhibitors, such as protease inhibitors and antifungal agents. One subject received efavirenz, a CYP3A4 inducer.

**PK analysis.** Descriptive statistics for plasma 1263W94 PK parameters are displayed in Table 2. There was a dose-proportional increase in plasma 1263W94 AUC_{ss}, C_{max}, and AUC_{24,ss} over the dose range tested, as displayed in Fig. 1. The slope estimates (and 90% CI) for plasma 1263W94 parameters were as follows: AUC_{ss}, 1.12 (0.98, 1.27); AUC_{24,ss}, 0.95 (0.84, 1.06); and C_{max}, 0.98 (0.85, 1.10).
TABLE 1. Demographic and baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value for dosing group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 mg t.i.d. (n = 11)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>37.0 (28.0, 57.0)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>7 (64)</td>
</tr>
<tr>
<td>Black</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>177.0 (170.0, 185.0)</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>79.1 (56.0, 105.5)</td>
</tr>
<tr>
<td>CD4(^+) cell count (cells/mm(^3))</td>
<td>110.0 (34.0, 256.0)</td>
</tr>
<tr>
<td>CDC classification</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>3 (27)</td>
</tr>
<tr>
<td>AIDS indicator</td>
<td>8 (73)</td>
</tr>
</tbody>
</table>

- Values for age, height, weight, and CD4\(^+\) cell count are given as median (minimum, maximum).
- Values for race and CDC classification are given as the number (percentage) of subjects with the particular characteristic.

TABLE 2. Plasma 1263W94 PK parameters\(^a\) at days 1 and 28

<table>
<thead>
<tr>
<th>Dosing group</th>
<th>AUC_{\text{day 1}} (\mu g h/ml)</th>
<th>AUC_{\text{day 28}} (\mu g h/ml)</th>
<th>C_{\text{max}} (\mu g/ml)</th>
<th>C_{\text{max,ss}} (\mu g/ml)</th>
<th>C_{\text{min,ss}} (\mu g/ml)</th>
<th>C_{\text{avg,ss}} (\mu g/ml)</th>
<th>T_{\text{max}} (h)</th>
<th>T_{\text{max,ss}} (h)</th>
<th>t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg t.i.d.(^c)</td>
<td>16.04 (10.56, 24.34)</td>
<td>15.58 (11.13, 21.80)</td>
<td>3.50 (2.57, 4.75)</td>
<td>3.98 (2.85, 5.56)</td>
<td>0.65 (0.35, 1.18)</td>
<td>1.95 (1.39, 2.73)</td>
<td>1.49 (0.50, 3.08)</td>
<td>2.00 (0.98, 3.02)</td>
<td>5.25 (3.99, 6.91)</td>
</tr>
<tr>
<td>200 mg t.i.d.(^c)</td>
<td>29.75 (19.01, 46.58)</td>
<td>23.51 (12.78, 43.32)</td>
<td>7.10 (4.94, 10.20)</td>
<td>6.55 (4.26, 10.06)</td>
<td>0.79 (0.24, 2.53)</td>
<td>2.94 (1.59, 5.41)</td>
<td>1.75 (1.00, 3.00)</td>
<td>1.75 (1.00, 3.00)</td>
<td>4.52 (3.62, 5.62)</td>
</tr>
<tr>
<td>400 mg t.i.d.(^c)</td>
<td>76.43 (52.65, 110.95)</td>
<td>74.47 (58.63, 94.60)</td>
<td>16.89 (12.78, 22.33)</td>
<td>18.48 (14.51, 23.45)</td>
<td>4.00 (2.97, 5.39)</td>
<td>9.31 (7.33, 11.82)</td>
<td>1.50 (1.00, 4.00)</td>
<td>1.50 (1.00, 2.00)</td>
<td>5.95 (4.35, 8.13)</td>
</tr>
<tr>
<td>600 mg b.i.d.(^c)</td>
<td>134.45 (77.02, 234.71)</td>
<td>147.19 (82.76, 261.77)</td>
<td>25.40 (15.68, 41.14)</td>
<td>31.21 (18.40, 52.94)</td>
<td>6.40 (3.06, 13.56)</td>
<td>17.47 (11.61, 26.30)</td>
<td>1.50 (1.00, 3.00)</td>
<td>1.50 (1.00, 3.00)</td>
<td>6.83 (4.62, 10.08)</td>
</tr>
<tr>
<td>900 mg b.i.d.(^c)</td>
<td>183.95 (140.11, 241.50)</td>
<td>209.69 (139.33, 315.57)</td>
<td>34.46 (29.41, 40.37)</td>
<td>41.01 (30.15, 55.76)</td>
<td>6.44 (3.06, 13.56)</td>
<td>17.47 (11.61, 26.30)</td>
<td>1.50 (1.00, 3.00)</td>
<td>1.50 (0.50, 3.00)</td>
<td>5.34 (4.36, 6.54)</td>
</tr>
<tr>
<td>1,200 mg b.i.d.(^c)</td>
<td>228.27 (132.61, 392.96)</td>
<td>225.41 (155.36, 327.05)</td>
<td>30.83 (21.78, 43.64)</td>
<td>41.26 (31.06, 54.80)</td>
<td>4.04 (1.58, 10.33)</td>
<td>18.78 (12.94, 27.26)</td>
<td>2.50 (1.00, 3.00)</td>
<td>1.50 (1.00, 4.00)</td>
<td>6.40 (4.53, 9.06)</td>
</tr>
</tbody>
</table>

- Values are geometric means (95% CI), except for T_{\text{max}} and T_{\text{max,ss}}.
- Values are medians (with ranges in parentheses).
- \(^{c}\) n = 10 for day 1; n = 11 for day 28.
- \(^{d}\) n = 12 for day 1; n = 6 for day 28.
- \(^{e}\) n = 6 for day 1; n = 5 for day 28.
1263W94 demonstrated linear PK, with steady-state plasma 1263W94 profiles predictable based on single-dose data as demonstrated by the ratio (and 90% CI) of AUCₙₙ to AUCₙₙ. The ratio of plasma 1263W94 AUCₙₙ to AUCₙₙ was close to 1, and the associated 90% CI was relatively balanced on either side of 1, for each of the doses administered.

The percentage of the dose that was excreted in urine as 1263W94 was minimal; on average, less than 3% of the parent compound was eliminated in urine. Semen 1263W94 concentrations increased with increasing doses. Median 1263W94 concentrations in semen (with the range and number of subjects given in parentheses) were as follows: for the 100-mg t.i.d. cohort, 1.67 μg/ml (0.30 to 3.53; n = 9); for the 200-mg t.i.d. cohort, 2.96 μg/ml (2.54 to 3.09; n = 4); for the 400-mg t.i.d. cohort, 8.41 μg/ml (3.62 to 25.57; n = 9); and for the 900-mg b.i.d. cohort, 11.85 μg/ml (3.42 to 21.77; n = 8). A semen 1263W94 concentration of 6.22 μg/ml was measured for a single subject in the 600-mg b.i.d. cohort.

Quantitation of HCMV in semen and whole blood. Figure 2 presents changes from baseline in semen HCMV amounts (based on plaque assays and PCR assays) for subjects in the main group at days 4 (when applicable), 7, 14, 21, and 28. Based on HCMV titers, subjects in the 200-mg t.i.d. (n = 5) and the 400-mg t.i.d. (n = 6) cohorts experienced the greatest decreases in HCMV amounts, with a mean decrease at day 28 of 3.7 log₁₀ PFU/ml (standard deviations [SD], 0.96 for the
200-mg t.i.d. cohort and 1.08 for the 400-mg t.i.d. cohort), while subjects in the 100-mg t.i.d. cohort (n = 7) experienced a mean (±SD) decrease of 2.9 (±0.79) log_{10} PFU/ml and subjects in the 600-mg b.i.d. (n = 6) cohort experienced a mean (±SD) decrease of 3.3 (±1.32) log_{10} PFU/ml. Quantitative reductions in semen HCMV DNA amounts and the differences between the cohorts were less pronounced than for HCMV amounts as measured by titers. At day 28, mean decreases from baseline in HCMV DNA amounts were 1.1 log_{10} copies/ml for the 100-mg t.i.d. cohort (n = 7), 1.5 log_{10} copies/ml for the 200-mg t.i.d. cohort (n = 6), and 1.3 log_{10} copies/ml for both the 400-mg t.i.d. (n = 5) and 600-mg b.i.d. (n = 2) cohorts.

The numbers (and percentages) of subjects in each cohort with ≥2-log_{10} unit reductions from baseline in semen HCMV titers at day 28 were as follows: 6 of 7 subjects (86%) in the 100-mg t.i.d. cohort, 5 of 5 subjects (100%) in the 200-mg t.i.d. cohort, 6 of 6 subjects (100%) in the 400-mg t.i.d. cohort, and 5 of 6 subjects (83%) in the 600-mg b.i.d. cohort. Only a single subject in each cohort had a ≥2-log_{10} unit reduction in semen HCMV DNA concentrations from baseline to day 28.

Only one subject, who received 1263W94 at 600 mg b.i.d., had quantifiable HCMV DNA in whole blood at baseline (4.81 log_{10} copies/ml). In this subject HCMV DNA amounts decreased to below the limit of detection by the PCR assay (<1.2 log_{10} copies/ml) by day 28.

**Detection of HCMV in the urine.** The numbers (and percentages) of subjects in each cohort whose qualitative HCMV cultures showed positive results at baseline and negative results in the last two on-treatment measurements (days 21 and 28) were as follows: 2 of 7 (29%) in the 100-mg t.i.d. cohort, 1 of 5 (20%) in the 200-mg t.i.d. cohort, 3 of 6 (50%) in the 400-mg t.i.d. cohort, and 2 of 6 (33%) in the 600-mg b.i.d. cohort.

FIG. 2. (a) Median concentration-time profiles of HCMV in semen, measured in PFU per milliliter by using plaque assays. Symbols: ○, 100 mg t.i.d. (n = 7); ■, 200 mg t.i.d. (n = 5); , 400 mg t.i.d. (n = 6); △, 600 mg b.i.d. (n = 6). (b) Median concentration-time profiles of HCMV DNA in semen, measured in log_{10} copies per milliliter by using PCR analysis. Symbols: ○, 100 mg t.i.d. (n = 7); ■, 200 mg t.i.d. (n = 4); , 400 mg t.i.d. (n = 5); △, 600 mg b.i.d. (n = 2).
HCMV sensitivity to 1263W94 by plaque reduction assay.

Ninety-two HCMV isolates were obtained from day-1, day-28, or day-56 cultures of semen and urine samples. One subject provided a day-11 semen sample, which was positive for HCMV and was included in the analysis. The 92 isolates analyzed were from 31 subjects in the 100-mg t.i.d., 200-mg t.i.d., and 400-mg t.i.d. cohorts and included 58 semen samples (24 from day 1, 1 from day 11, 15 from day 28, and 18 from day 56) and 34 urine samples (13 from day 1, 9 from day 28, and 12 from day 56).

The median 50% inhibitory concentration (IC$_{50}$) of 1263W94 for the clinical isolates was 0.27 μM (range, 0.05 to 0.88 μM). There were no significant differences in the median IC$_{50}$ between the day-1 samples and the day-28 or day-56 samples. The median IC$_{50}$ for wild-type strain AD169, determined from 10 separate plaque reduction assays, was 0.55 μM (range, 0.23 to 0.78 μM).

Because the clinical isolates tested might contain mixed populations of drug-susceptible virus and treatment-emergent drug-resistant virus selected by exposure to 1263W94, additional assays were performed to determine the level of resistant virus in a mixed viral population necessary to produce an increase in the IC$_{50}$. Mixtures of wild-type AD169 and the 1263W94-resistant strain 2916 were assayed for 1263W94 susceptibility by using plaque reduction assays. IC$_{50}$, did not increase with a mixture containing 10% resistant virus but increased 3.3- and 16-fold with mixtures containing 25 and 50% resistant virus, respectively. These results suggested that emergence of 1263W94-resistant variants comprising as little as 25% of the viral population would have been detected in plaque reduction assays of the clinical isolates.

Safety and tolerability. The majority of subjects enrolled in the study, including 60 of 62 subjects (97%) who received 1263W94 and 10 of 16 subjects (63%) who received the placebo, experienced at least one adverse event during the study period. Most of the subjects reported adverse events that were neurological, such as taste disturbances and headache, or gastrointestinal, such as diarrhea and nausea. Adverse events reported by ≥10% of subjects in either the 1263W94 or the placebo group, and the numbers (and percentages) of subjects reporting the event in the 1263W94 and placebo groups, respectively, were as follows: taste disturbances, 51 (82%) and 3 (19%); headache, 13 (21%) and 3 (19%); diarrhea, 16 (26%) and 2 (13%); nausea, 14 (23%) and 2 (13%); rash, 12 (19%) and 1 (6%); pruritus, 12 (19%) and 1 (6%); fever, 7 (11%) and 0; exacerbation of fatigue, 6 (10%) and 2 (13%); vomiting, 5 (8%) and 2 (13%); constipation, 0 and 2 (13%); and upper respiratory tract infection, 2 (3%) and 2 (13%). Diarrhea and taste disturbances appeared to be dose related. After approximately 7 to 12 days of therapy, five subjects receiving 1263W94 pre maturely discontinued the study due to a drug-related diffuse maculopapular rash of moderate (grade-2) intensity. Two of these subjects received 200 mg t.i.d., and one each received 600, 900, and 1,200 mg b.i.d. Four of these subjects had a history of allergic reaction to other drugs. After discontinuation of the study drug, the rash resolved within approximately 1 to 4 days without sequelae.

Two serious non-drug-related adverse events were reported during the study. The first event was cholecystitis, for which the subject underwent a cholecystectomy. The second event was a single episode of pulmonary thromboembolism. The symptoms of pulmonary thromboembolism (dyspnea and chest pain) resolved within 2 weeks of hospital admission and initiation of appropriate therapy. Both events occurred in subjects who received the placebo.

Clinical laboratory values for subjects who received 1263W94 and subjects who received the placebo were not significantly different, and there were no dose-related trends in clinical laboratory values. Decreases in lymphocytes and increases in total protein were consistent with the HIV-infected status of the subjects, and modest decreases in hemoglobin were consistent with the phlebotomy requirements of the protocol. Hemoglobin values increased to screening values by the 4-week poststudy visit.

DISCUSSION

There is a need to develop effective and safe oral therapies for the treatment and prevention of HCMV disease. 1263W94 is a benzimidazole riboside with potent and selective inhibition of HCMV in vitro and with limited cytotoxic and toxicologic effects based on in vitro and in vivo screenings (1). Pre-clinical studies have indicated that 1263W94 has good oral bioavailability (9).

This study was designed to evaluate the PK, anti-HCMV activity, safety, and tolerability of 1263W94 administered as multiple oral doses to HIV-infected subjects with asymptomatic HCMV shedding in urine and semen over a 28-day treatment period. Anti-HCMV activity was evaluated by measuring HCMV titers in semen (5, 10) and by measuring HCMV DNA amounts in semen and whole blood by PCR. Urine cultures were not used for quantitation of HCMV, because the urinary tract is a closed system, and variations in urine volume may affect accurate quantitation of HCMV.

Semen HCMV titration has proven useful in demonstrating in vivo anti-HCMV activity and in selecting clinically useful doses of HCMV therapies. For example, cidofovir was more effective at reducing semen HCMV titers at a weekly dose of 5.0 mg/kg of body weight than at a weekly dose of 3.0 mg/kg (10), and the higher dose was also more effective in clinical trials (11, 12).

Over 28 days of dosing, 1263W94 demonstrated in vivo anti-HCMV activity in semen at all of the dosage regimens tested (100, 200, and 400 mg t.i.d., and 600 mg b.i.d.), with mean reductions in semen HCMV titers of 2.9 to 3.7 log$_{10}$ PFU/ml among these four regimens. The lowest dosage regimen appeared to have less anti-HCMV activity than the three higher dosage regimens; however, the reductions in HCMV titers for all of the 1263W94 dosage regimens tested compare favorably with results reported for the approved doses of cidofovir (5 mg/kg) (10).

A greater change from baseline in HCMV load following administration of 1263W94 was measurable by culture using plaque titration than by quantitative PCR. An explanation could be that synthesis of DNA continues to some degree in the presence of 1263W94 but that intact viable virus is not produced. Thus, the antiviral effect of 1263W94 could initially
be more evident by culture of viable virus than by quantitation of HCMV DNA synthesis. Also, the quantitative reduction in viral DNA may lag behind plaque reduction. If we had continued to assay viral DNA in semen at weeks 5 and 6 instead of stopping at 28 days, we might have seen greater log reductions in HCMV DNA concentrations.

Although relatively high concentrations of 1263W94 were detected in semen, the anti-HCMV effect in semen as measured by plaque assay is not attributable to drug carryover into viral cultures, as shown by the following observations. (i) Semen HCMV titers decreased progressively from day 1 to day 28 (see Fig. 2); a carryover effect should have been equally apparent in all sequential samples. (ii) HCMV DNA levels in semen also decreased over the 28-day treatment period. The PCR assay is a direct measure of viral DNA at the time of specimen collection and is not subject to inhibition by 1263W94 during the assay.

There was a dose-proportional increase in plasma 1263W94 AUC_0–t, C_{max}, and AUC_24–ss over the dose range tested. 1263W94 demonstrated linear PK, with steady-state plasma profiles predictable based on single-dose data. Several of the concomitant medications that subjects received for treatment of HIV and opportunistic infections were inhibitors, inducers, and/or substrates of CYP3A4, the isoenzyme primarily responsible for 1263W94 metabolism. The effects of these drugs on 1263W94 PK were not assessed.

1263W94 was generally safe and reasonably well tolerated during the 28 days of dosing. Six subjects prematurely discontinued the study drug due to adverse events: five cases of rash and one of sinusitis. Two subjects, both receiving the placebo, reported serious non-drug-related adverse events during the study. Taste disturbance was the most frequently reported adverse event. Taste disturbance, diarrhea, nausea, rash, pruritus, and fever were reported by a higher percentage of subjects receiving 1263W94 versus the placebo, and taste disturbance and diarrhea appeared to be dose related. Overall, 1263W94 showed a favorable profile with regard to safety, tolerability, PK, and anti-HCMV effect.

The absence of resistance in isolates obtained at day 28 of treatment (or 28 days later) is encouraging but is similar to previous data for ganciclovir and cidofovir (2, 4). In order to evaluate the risk of developing resistance, it would be necessary to assess IC_{50} for isolates from patients receiving 1263W94 for ≥90 days (4, 8).

The oral bioavailability of 1263W94, the duration and magnitude of its antiviral effect, and the lack of dose-limiting toxicity make it very attractive as a potential anti-HCMV therapeutic and prophylactic agent. The reductions in HCMV titers for all of the 1263W94 dosage regimens tested compare favorably with results reported for the approved anti-HCMV agents. In addition, the relatively benign toxicity profile of 1263W94 and the specific absence of nephrotoxicity or myelotoxicity suggest a potential role for this agent for patients receiving solid-organ and bone marrow transplants as well as for patients with HIV.

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