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Multiple-Dose Escalation Study of the Safety, Pharmacokinetics, and Biologic Activity of Oral AMD070, a Selective CXCR4 Receptor Inhibitor, in Human Subjects▽†

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AMD070 is an oral CXCR4 antagonist with in vitro activity against X4-tropic human immunodeficiency virus type 1. Thirty fasting healthy male volunteers received oral doses of AMD070 ranging from a single 50-mg dose to seven 400-mg doses given every 12 h (q12h). Nine subjects received a 200-mg dose during fasting and prior to a meal. Subjects were monitored for safety and pharmacokinetics. AMD070 was well tolerated, without serious adverse events. Transient headaches (13 subjects) and neurocognitive (8 subjects) and gastrointestinal (7 subjects) symptoms were the most common complaints. Seven subjects had sinus tachycardia, and two were symptomatic. AMD070 plasma concentrations peaked 1 to 2 h after patient dosing. The estimated terminal half-life ranged from 11.2 to 15.9 h among cohorts. Dose proportionality was not demonstrated. Less than 1% of the drug appeared unchanged in the urine. Food reduced the maximum concentration of drug in serum and the area under the concentration-time curve from 0 to 24 h by 70% and 56%, respectively ($P \leq 0.01$). A dose-dependent elevation of white blood cells (WBC) demonstrated a maximum twofold increase over baseline (95% confidence interval, 2.0- to 2.1-fold) in an E_{max} model. In healthy volunteers, AMD070 was well tolerated and demonstrated mixed-order pharmacokinetics, and food reduced drug exposure. AMD070 induced a dose-related elevation of WBC which was attributed to CXCR4 blockade. Using leukocytosis as a surrogate marker for CXCR4 inhibition, this dose-response relationship suggests that the doses used in this study were active in vivo, though not maximal, throughout the dosing interval. Trough concentrations with the 400-mg dose q12h exceeded the antiviral in vitro 90% effective concentration of AMD070.

Despite the positive life-prolonging impact of current antiretroviral therapy on human immunodeficiency virus (HIV)-infected patients, viral resistance to conventional reverse transcriptase and protease inhibitors has limited long-term treatment success for many patients. As antiviral development evolved to manage this therapeutic failure, a new group of antiviral agents, which target the process of viral entry, emerged. The complex series of steps leading to viral fusion and entry into host cells provide several potential targets for drug therapy. Although initial interactions of the viral envelope are with the CD4 receptor, binding to CD4 causes a conformational change in the envelope protein, gp120, allowing it to bind to a cell surface chemokine coreceptor (CXCR4 or CCR5) to further facilitate fusion of viral and cell membranes (1). Viruses that utilize CXCR4 as their coreceptor are the T-lymphocyte-tropic X4 strains, while macrophage-tropic, R5 strains use CCR5. Antagonists to each coreceptor are being developed as potential HIV entry inhibitors.

A previously investigated small-molecule CXCR4 antagonist, AMD3100, demonstrated anti-HIV activity in vitro and in the SCID-hu Thy/Liv mouse model (3). During a phase II study, 40 HIV-infected subjects were given a 10-day continuous intravenous infusion of AMD3100 (4). One participant with pure X4 virus had a 0.87-$\log_{10}$ reduction in HIV RNA, and 9 of 16 participants with dual-tropic viruses (harboring both X4 and R5 viruses) had reductions in the level of X4 virus to below the limit of detection following treatment. This antiviral effect legitimizes the use of CXCR4 inhibition as an antiretroviral strategy. In clinical studies, AMD3100 was also found to produce a dose-dependent leukocytosis attributed to CXCR4 antagonism. Despite promising anti-HIV effects, the desire to use an orally bioavailable compound led to the halt of AMD3100 development for treatment of HIV infection.

AMD070, a new chemical entity, is a CXCR4 antagonist with a 50% inhibitory concentration similar to that of

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TABLE 1. Study schema

Phase of study	Cohort	No. of subjects	Dose	Frequency	No. of doses
Single dose	A	3	50	Once	1
	B	3	100	Once	1
	C	3	200	Once	1
	D	3	400	Once	1
Multiple dose	F	6	100	q12h	7
	G	6	200	q12h	7
	I	6	400	q12h	7
Fed/fasted	H	6[a]	400	Once (with food)	1
	K	9[b]	200	Twice (with and without food)	2

[a] Included for safety analysis only. All subjects were also admitted into other cohorts.
[b] Includes five subjects who were admitted into other cohorts.

AMD3100 (2.3 ng/ml and 1.5 ng/ml, respectively) against HIV-1 NL4.3 in MT-4 cells. The protein-binding adjusted 90% effective concentration (EC_{90}) for AMD070 (assuming 90% protein binding) against HIV-1 in MT-4 cells is 44 ng/ml (AnorMED, Inc.).

Oral bioavailabilities of 20% and 80% have been calculated for the rat and dog, respectively. Elimination occurred from plasma in a biexponential manner, with rapid initial half-lives ($t_{1/2}$) of 1.4 and 0.7 h for the rat and dog, respectively, and a longer terminal elimination $t_{1/2}$, of approximately 10 h, for both species (AnorMED, Inc.).

Given that prior clinical studies demonstrating an antiretroviral effect suggested CXCR4 antagonism as a potentially viable therapeutic strategy for HIV therapy and the preclinical efficacy and safety of AMD070, we designed this phase I clinical investigation to describe the safety, pharmacokinetics, and pharmacodynamic effects of AMD070 administered orally to healthy human subjects.

(Partial results were presented previously at the 5th International Workshop on Clinical Pharmacology of HIV Therapy, Rome, Italy, April 2004 [11], and the XV International AIDS Conference, Bangkok, Thailand, July 2004 [12].)

MATERIALS AND METHODS

Study design and population. This was a phase I, first-in-human, open-label, single- and multiple-dose escalation study of AMD070 in healthy male volunteers conducted at two research sites, namely, Johns Hopkins University and the University of Washington, as part of ACTG Study A5191. Reproductive toxicity data from preclinical studies were not available to warrant enrollment of uninfected women. Subjects were healthy, HIV-seronegative men between the ages of 18 and 55 with no active medical illness by history, physical, or laboratory evaluation. No concomitant medications were allowed 2 weeks prior to and throughout the study period. Dose escalation occurred only if the preceding dose was determined to be safe and well tolerated. Four dosage cohorts, A to D, with three volunteers each received a single oral dose of AMD070 at escalating dosages (50, 100, 200, and 400 mg). Following this, three dosage cohorts (F, G, and I) with six volunteers each received AMD070 every 12 h (q12h) for a total of seven doses at escalating dosages (100, 200, and 400 mg).

Six volunteers from the dose escalation cohorts (A to D, F, G, and I) were also readmitted and received a single 400-mg AMD070 dose administered with a standardized breakfast, forming cohort H (Table 1). Data from these subjects could not be used to determine the effect of food on AMD070 absorption due to a lack of dose proportionality. Therefore, nine volunteers (including five from prior cohorts) were admitted on two separate occasions to receive a single 200-mg AMD070 dose administered either while fasting or with a standardized breakfast, forming cohort K (Table 1).

The protocol was approved by the institutional review boards at both research sites, and all study participants provided written informed consent prior to enrollment. This research complied with all relevant federal guidelines and institutional policies.

Study procedures. Prospective subjects were screened by medical history, physical exam, electrocardiogram, and laboratory testing to determine study eligibility. Eligible subjects were admitted to the general clinical research center for either 2 nights (single-dose cohorts) or 5 nights (multiple-dose cohorts). In the first four single-dose cohorts, three subjects each took an oral dose of AMD070 (range, 50 to 400 mg) following an 8-hour overnight fast and continued fasting until 1 h post-AMD070 administration. Blood was collected for AMD070 concentration analysis according to the following schedule: before dose administration and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h after dosing. Starting with cohort C, blood samples for total white blood cell (WBC) counts and $CD4^+$ and $CD34^+$ cell counts were also collected before dose administration and 2, 4, 6, 8, and 24 h after dosing. Subjects in the multiple-dose cohorts were dosed q12h for seven total doses. Morning doses were administered following an 8-hour fast, and subjects continued fasting until 1 h post-dose administration, except for the fed cohort. Blood sampling occurred following the final dose (72 h after the initial dose) and followed the same schedule as that outlined above. Urine was collected for a 24-h period following the last dose.

To assess the effect of food, nine subjects (cohort K) were admitted to the general clinical research center on two separate occasions to receive a single 200-mg dose of AMD070, either while fasting or within 30 min of starting a standardized, low-fat breakfast of eggs, toast, and beverages (excluding grapefruit juice). Following each dose, blood was collected for AMD070 concentration analysis as described above, with an additional 48 h post-dosing sample.

Safety monitoring occurred throughout the inpatient stay, with frequent observations for adverse events and continuous cardiac telemetry. Blood and urine samples were collected for further safety evaluation. Subjects were discharged on the morning following administration of the single or final drug dose and returned 2 weeks later for safety evaluation, including medical history, targeted physical exam, electrocardiogram, and laboratory testing.

AMD070 assay. Concentrations of AMD070 in plasma and urine were determined by high-performance liquid chromatography-mass spectrometry (MS), using validated methods. Validations included assessments of linearity; within-run and between-run precision and accuracy; selectivity; long-term (frozen), short-term (room temperature), autosampler, freeze-thaw, and processed sample stability; extraction efficiency; carryover; the effect of dilution; and potential over-the-counter and HIV drug interferences. The validated calibration range was from 0.5 to 500 ng/ml for both plasma and urine and was selected based on linearity ($R^2 > 0.95$) and on accuracy and precision criteria for the validation runs. The acceptance criteria for accuracy and precision were 15% (20% at the lower limit of quantitation) deviation and an average coefficient of variance (CV) of ≤15% (≤20% at the lower limit of quantitation). Actual within-run precision and accuracy for the validation runs ranged from 3.7 to 14 (%CV) and −11 to 15 (% deviation). Between-run precision and accuracy ranged from 6.6 to 11 (%CV) and −5.2 to 12 (% deviation). The maximum validated dilution factor was 100-fold. Sample runs were accepted or rejected based on results obtained for quality control samples included in each run: at least 67% (e.g., six of nine samples) of the quality control samples were required to be within 15% of their respective nominal values. The coefficient of determination (R^2) must be >0.95.

Standard calibrators, quality control samples, and study samples were prepared and analyzed in an identical manner. Briefly, samples (100 μl) were heated for 30 min at 57°C, followed by the addition of 50 μl of a 0.5-mg/ml internal standard solution (AMD11025, a structural analog of AMD11070) and 50 μl of a 1 N sodium hydroxide (NaOH) solution. After mixing the samples briefly, 1.0 ml of methyl *tert*-butyl ether was added, and the samples were vortexed (10 min) and then centrifuged (3 min, >10,000 rpm). Samples were then frozen at ≤−60°C for approximately 60 min. The methyl *tert*-butyl ether layer was decanted into a second tube and evaporated to dryness in a water bath at 30°C under nitrogen. Following reconstitution in 200 μl of 5%-95%-0.1% acetonitrile-water-trifluoroacetic acid, the samples were analyzed by reversed-phase high-performance liquid chromatography with MS/MS detection (mobile phase, 7%-93%-0.1% acetonitrile-water-trifluoroacetic acid; flow rate, 0.5 ml/min; and run time, 4.5 min). Detection by MS/MS incorporates an electrospray interface in positive-ion mode.

PK analysis. Noncompartmental analysis was performed using WinNonlin Professional software (version 5.0.1; Pharsight Corp., Cary, NC). Concentration-time curves were plotted. Individual pharmacokinetic (PK) parameters were calculated using all available time points and then summarized by dose cohort, and they included time to maximum concentration (T_{max}), maximum concentration (C_{max}), concentrations 12 h (C_{12}) and 24 h (C_{24}) following dosing, terminal elimination $t_{1/2}$, areas under the concentration-time curves (using the log-linear

trapezoidal rule) from 0 to 12 h (AUC_{0-12}) and from 0 to 24 h (AUC_{0-24}) following the dose and extrapolated to infinity ($AUC_{0-\infty}$), total apparent oral clearance (CL_T/F), renal apparent oral clearance (CL_R/F), and apparent volume of distribution (V/F).

The Kruskal-Wallis test was used to test for any differences in point estimates among dose cohorts, with dose normalization where appropriate. Where the Kruskal-Wallis test was statistically significant, the Jonckheere-Terpstra test for ordered differences among groups was used to test for deviations from dose proportionality, using dose-normalized values (parameter values divided by dose) for C_{max}, AUC_{0-inf}, AUC_{0-12}, and C_{12}, and to test for dose-dependent differences in CL_T/F, CL_R/F, V/F, and $t_{1/2}$ (6). Within each dose level (100, 200, and 400 mg), the single- versus multiple-dose PK parameters were compared using the Wilcoxon rank sum test. The effect of food was tested using the Wilcoxon signed-rank test to compare the PK parameter estimates between the fasted and fed periods for cohort K subjects. All statistical tests were performed using SPSS (version 9.0.1; SPSS, Inc., Chicago, IL); the significance level for all tests was set at <0.05 and was two-sided, with no adjustment for multiple comparisons.

Pharmacodynamic analysis. Leukocytes and leukocyte subsets were plotted against time and against the AMD070 concentration. Leukocyte and leukocyte subset ratios for each observation were calculated as the values for the leukocyte and subset populations following AMD070 dosing divided by the baseline leukocyte and subset values. For WBC, $CD4^+$ T-cell, and $CD34^+$ stem cell ratios, the C_{max}, C_{24}, AUC_{0-12}, and AUC_{0-24} were calculated. Deviations from expected parameter values consistent with no pharmacodynamic effect (1.0-fold change in C_{max} and C_{24}, 12-unit-hour change for AUC_{0-12}, and 24-unit-hour change for AUC_{0-24}) were evaluated using Wilcoxon rank sum tests. Associations between the magnitude of response and total dose exposure were evaluated using the Jonckheere-Terpstra test, with total dose as the ordered variable.

Values for the WBC ratio (response) and AMD070 concentration (dose) were fit to the following dose-response sigmoid maximum effect model, using WinNonlin Professional software (version 5.0.1; Pharsight Corp., Cary, NC): WBC ratio = $E_{max} \cdot [AMD070/(EC_{50} + AMD070)]$, where E_{max} is the estimate of the maximum WBC ratio (maximal effect) and EC_{50} is the AMD070 concentration associated with the half-maximal WBC ratio (half-maximal effect). The maximum WBC ratio for each subject was fit to the dose, AUC_{0-24}, and C_{max}. All times for all subjects were also used to fit the WBC ratio at each sampling time against each AMD070 concentration. These models were also fit with inclusion of a sigmoidicity (Hill) coefficient and compared to the simpler model, using the correlation coefficient, CV, and both the Aikake and Schwartz information criteria. Because the effect outcome was a ratio, models with y-intercept terms (E_0) were not evaluated.

RESULTS

Subjects. A total of 34 male subjects participated in the study, including 11 who participated in two cohorts (Table 1). Another subject was enrolled but never received study medication because of cardiac arrhythmias (frequent premature ventricular contractions) noted at baseline. Of those who received study medication, 30 subjects were enrolled in the study for fasting-only dosing, among which 12 subjects, 3 in each of four dose cohorts, each received a single oral dose of AMD070 (cohorts A, B, C, and D received 50, 100, 200, and 400 mg, respectively) and 18 subjects, 6 in each of three dose cohorts, each received seven doses of AMD070 q12h (cohorts F, G, and I received 100, 200, and 400 mg, respectively). Six subjects returned to comprise cohort H, although their PK results are not included (see below). Additionally, nine subjects were enrolled in the fed versus fasted comparison (cohort K), including five subjects from the previous cohorts. Subjects included 18 (53%) African Americans, 15 (43%) whites, and 1 (3%) Hispanic. At study entry, the ages ranged from 18 to 54 years (median, 40 years) and weights ranged from 53 kg to 103 kg (median, 80 kg).

Safety. Subjects who participated in multiple cohorts of the study were considered unique subject enrollees in each cohort for safety purposes. Thirty-one of 45 subject enrollees (69%) had a new symptom or laboratory abnormality during the inpatient phase of the study (26 had symptoms and 15 had a laboratory abnormality; 10 had both). All adverse events were mild (grade 1), except for one subject with an asymptomatic elevated lipase level (grade 3), seven subjects with moderate (defined as analgesia-requiring) headaches (grade 2), and one subject with moderate diarrhea (grade 2); all abnormalities except for the one grade 3 lipase elevation resolved during the study or in follow-up (Table 2). No serious adverse events were reported, and no subject missed a dose or was discontinued from the study because of adverse effects. Thirteen subjects complained of headache (six of which were also associated with jaw, eye, or ear pain), the most common complaint during the study. Eight subjects reported nine distinct anatomic clusters of gastrointestinal symptoms, which included gas, cramps, abdominal distention/pain, loose stools, and/or heartburn. A variety of neurocognitive symptoms were reported by seven subjects (one with two distinct symptoms, namely, perioral paresthesias and drowsiness).

Seven subjects had electrocardiographic evidence of sinus tachycardia, symptoms consistent with tachycardia, or both. Two of these subjects, both of whom were in multiple-dose cohorts, reported associated symptoms (lightheadedness and either palpitations or a flushed sensation) on some, but not all, occasions of tachycardia. For the multiple-dose subjects, the electrocardiographically documented tachycardia was characterized by a peak rate from 100 to 144, which almost always followed a positional change to the standing position and occurred over several days. One subject, in contrast, had one episode of sinus tachycardia with a rate of 168 while sitting. There were no cases of ventricular tachycardia. The PR intervals, QTc intervals, and QRS durations did not vary by more

TABLE 2. Adverse events

Adverse event category	No. of subjects with event at grade[a]		
	1	2	3
Symptoms			
Headache[b]	6	7	
Gastrointestinal symptoms	8	1	
Neurologic/cognitive symptoms[c]	8		
Palpitations/lightheadedness	2		
Other[d]	7		
Laboratory abnormalities			
Sinus tachycardia (electrocardiogram)[e]	7		
Lipase increase	3		1
Total bilirubin increase	2		
Liver transaminase increase	2		
Uric acid increase	1		
Phosphate decrease	1		

[a] Numbers are mutually exclusive within each category. The data include only new inpatient adverse events during the study and exclude new outpatient events during follow-up study of patients off the drug. No grade 4 events were recorded.
[b] Six headaches were also associated with jaw, ear, eye, or tooth pain.
[c] Includes three drowsiness cases (one also with perioral paresthesias), insomnia, feeling intoxicated, a buzzing sensation, and dizziness.
[d] Includes backache, stiffness, skin papules, conjunctivitis, two upper respiratory infections, and sweats.
[e] Includes only two subjects with symptoms consistent with tachycardia.

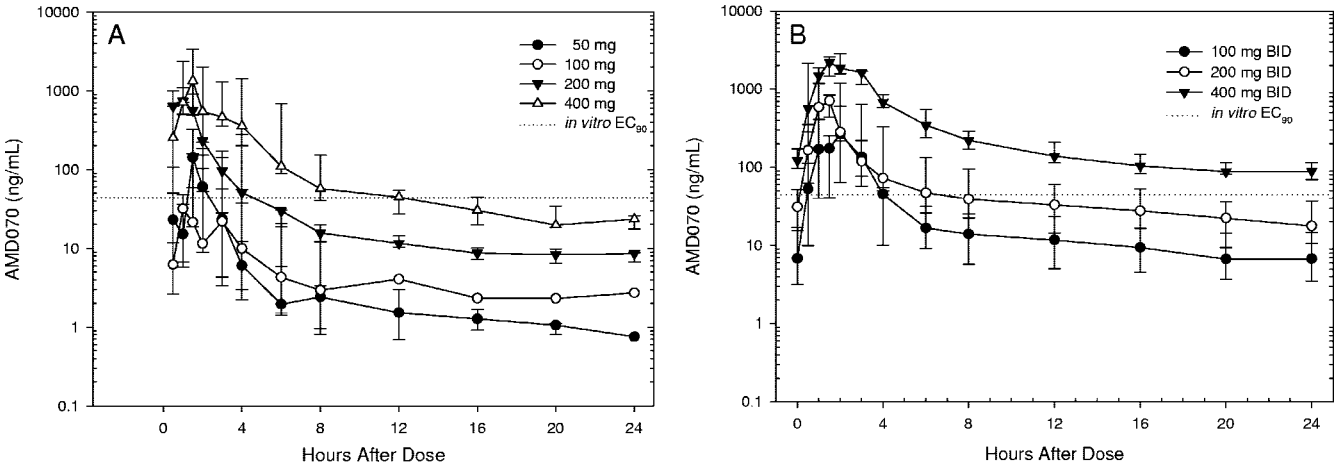

FIG. 1. Single (A)- and multiple (B)-dose cohort concentration-versus-time plots. The single-dose pharmacokinetics of AMD070 (median and IQR) are shown by dose cohort. Both figures use the same scale.

than 0.03 ms, 0.04 ms, and 0.02 ms, respectively. ST or T wave morphology did not change significantly compared to baseline.

The most commonly observed laboratory abnormality during the inpatient phase was an expected elevation in WBC count seen for all subjects for whom WBC data were collected (see details in "Pharmacodynamics" below). Among unexpected laboratory changes, we noted lipase elevations in four subjects, which normalized by the 2-week safety visit in three of them. In the fourth subject, this elevated lipase was grade 3 (134 mg/dl) and dropped only to grade 1 (87 mg/dl) during follow-up. All other laboratory abnormalities were reversible and mild (Table 2). Although creatine phosphokinase (CPK) was the most common laboratory abnormality noted, it did not newly arise in any subject during the inpatient AMD070 dosing phase of the study. It was elevated prior to dosing in six subjects, resolving fully in five of them during the inpatient observation for AMD070 dosing, and newly arose in five subjects at or after the 2-week follow-up visit, ranging from grades 1 to 3. There were also five subjects with elevated glucose, although none of the samples were collected in the fasting state. The number of adverse events per subject, combining both laboratory changes and symptoms, was not dose related for either individual doses or total dose (Jonckheere-Terpstra test; exact $P = 0.9$ and 0.3, respectively), nor was it correlated with C_{max} or AUC_{0-24} (Spearman correlation coefficient, 0.64 and 0.51, respectively).

Pharmacokinetics. AMD070 concentration-versus-time plots for the 30 subjects in fasted cohorts demonstrate parallel terminal elimination slopes (Fig. 1). Typically, the rate of decay over time demonstrated biexponential logarithmic decay. The interindividual variability in concentration was greater during the absorption and distribution phases, where concentrations among cohorts often overlapped. In the terminal elimination phase, dose cohorts were discretely separate. Concentrations peaked between 1 and 2 h after dosing, and T_{max} did not differ among cohorts (Kruskall-Wallis test; $P = 0.27$).

For the AMD070 C_{max}, $AUC_{0-\infty}$, and AUC_{0-12}, there was a high degree of variability within dose cohorts (Table 3), and there appeared to be disproportionate increases in AUC and C_{max} with increasing doses (Fig. 2). For both single- and multiple-dose cohorts, statistically significant dose-related increases were still present after dose adjustment of the AUC (Jonckheere-Terpstra test; $P < 0.001$), inconsistent with dose proportionality. For the dose-adjusted median concentration 12 h after dosing (C_{12}), statistically significant dose-related increases were also seen for both single- and multiple-dose cohorts, even after dose adjustment (Jonckheere-Terpstra test; $P < 0.02$). The magnitude of change was a 4.8-fold AUC increase and 4.2-fold C_{12} increase with only a doubling of dose, from 200 mg to 400 mg, for the multiple-dose cohorts. Unlike AUC and C_{12}, perhaps because of large variability, the dose-adjusted C_{max} was not significantly different across dose levels for both single- and multiple-dose cohorts (Kruskall-Wallis test; $P = 0.092$ and 0.36, respectively).

The median estimated terminal $t_{1/2}$ ranged from 7.6 to 12.6 h for the single-dose cohorts and from 11.2 to 15.9 h for the multiple-dose cohorts, without significant differences among either single- or multiple-dose cohorts (Kruskall-Wallis test; $P = 0.16$ and 0.58, respectively) (Table 3). CL_T/F decreased fourfold with increasing doses, from the 50-mg single-dose cohort to the 400-mg single- and multiple-dose cohorts ($P < 0.01$). V/F also decreased fourfold with increasing doses, from the 50-mg cohort to the 400-mg single- and multiple-dose cohorts ($P \leq 0.05$). A small amount of AMD070 was recovered unchanged in the urine, with a median of 0.91% of the administered dose (interquartile range [IQR], 0.57% to 1.76%). CL_R/F did not change with increasing doses ($P = 1.0$) and had an estimated median (IQR) of 0.114×10^{-2} liter/h (0.075×10^{-2} to 1.36×10^{-2}), representing a median (IQR) of 0.96% (0.53% to 1.68%) of CL_T/F.

For the multiple-dose cohorts, the median C_{12} values were 5.7-fold (100 mg), 2.8-fold (200 mg), and 3.0-fold (400 mg) greater than the corresponding values for the single-dose cohorts; however, the difference was statistically significant only for the 400-mg dose cohort comparison ($P = 0.024$). This is consistent with dose accumulation with 12-h dosing (Fig. 1; Table 3). No other PK parameters, including T_{max}, C_{max}, AUC, V/F, CL_T/F, and $t_{1/2}$, were statistically different between single- and multiple-dose cohorts ($P > 0.05$). AMD070 concentrations 12 h after the seventh AMD070 dose (C_{12}) were greater than

concentrations immediately prior to the seventh dose for all but two subjects in the multiple-dose cohorts, by a median ratio of 1.24 (IQR, 1.06 to 1.53), suggesting that a steady state had not yet been achieved.

Six subjects from fasted cohorts A, B, and C were readmitted to receive a 400-mg dose of AMD070 immediately preceding a large breakfast (fed cohort H). Because dose proportionality was not shown in the dose escalation cohorts and because cohort H subjects each received a different dose in earlier fasted cohorts, these subjects' PK parameter estimates could not be dose adjusted to allow a fed versus fasted comparison. Accordingly, their PK results are not presented; however, they are included in the adverse event summary above. For cohort K, the fed and fasted doses were both 200 mg, thus allowing for comparison without the need for dose adjustment. The addition of food prior to dosing resulted in a median (IQR) decrease of 70% (58% to 78%) in C_{max} ($P = 0.008$) and of 56% (34% to 70%) in AUC_{0-24} ($P = 0.01$) compared to fasted dosing. T_{max} increased 167% (66% to 250%) with the addition of food ($P = 0.007$). There was no effect of food on terminal $t_{1/2}$ ($P = 0.68$).

The cohort receiving the highest dose, i.e., 400 mg q12h, reached a median peak concentration of 2,305 ng/ml (IQR, 1,944 to 3,069; range, 1,675 to 4,553) and a median concentration 12 h after the PK dose (C_{12}) of 137 ng/ml (IQR, 115 to 208; range, 102 to 287) (Table 3). The 12-hour postdose concentrations ranged from 2.3 to 6.5 times the protein-binding adjusted in vitro EC_{90} in MT-4 cells (44 ng/ml) (1).

Pharmacodynamics. Leukocytosis followed AMD070 dosing in all subjects, ranging from 1.3-fold (50-mg single-dose subject) to 2.9-fold (400-mg single-dose subject) above baseline, with a peak between 2 and 4 h following dosing (Fig. 3). The median magnitudes of this WBC count elevation for the multiple-dose cohorts were 1.8-fold (100 mg q12h), 1.7-fold (200 mg q12h), and 2.1-fold (400 mg q12h) ($P = 0.03$). Elevations of similar magnitude and temporal pattern were seen across all leukocyte subsets tested, which included neutrophils, lymphocytes, monocytes, basophils, eosinophils, $CD4^+$ T cells, and $CD34^+$ stem cells (data not shown). For subjects in the multiple-dose cohorts, the WBC ratio still remained above baseline 24 h following the final dose (median, 1.4 times baseline values [range, 1.0 to 2.1 times baseline values]).

Absolute CD4 cell count changes were similar in time course to the leukocytosis, with peak increases observed 2 h after dosing. Unlike the leukocytosis, however, CD4 cell counts dropped below baseline 24 h after dosing. The maximum increase in CD4 concentration relative to baseline was smaller in the multiple-dose cohorts (F, G, and I), with a median (IQR) 2.1-fold (1.9- to 2.7-fold) increase for single-dose cohorts (only data for cohorts C and D were available) but only a 1.4-fold (1.1- to 1.6-fold) increase for multiple-dose cohorts ($P = 0.001$). The CD4 cell count 24 h after dosing dropped below baseline in the multiple-dose cohorts, to a median (IQR) of 80% (73% to 84%) ($P = 0.08$) of baseline levels. Given the complex pattern of a CD4 cell increase early after dosing with a subsequent decrease below baseline at 24 h, we evaluated the overall time-averaged effect on the CD4 cell count over a 12-hour or 24-hour dosing interval by calculating the AUC_{0-12} and AUC_{0-24}. The CD4 cell AUC_{0-24} for the multiple-dose cohorts was a median (IQR) of 22.5 unit-hours (17.0 to 24.2

TABLE 3. PK summary by study cohort (fasting)

Cohort	Dose (mg)	Median (IQR)								
		T_{max} (h)	C_{max} (ng/ml)	Predose C^a (ng/ml)	C_{12} [b] (ng/ml)	$AUC_{0-\infty}$ or AUC_{0-12} [c] (h · ng/ml)	CL/F (liter/h)	V/F (liter)	$t_{1/2}$ (h)
Single-dose fasted cohorts									
A	50	1.5 (1.5–2.0)	159 (59–185)		1.5 (0.7–3.0)	216 (129–369)	0.231 (0.135–0.386)	3,620 (1,496–7,166)	10.8 (7.7–12.9)
B	100	1.5 (1.5–4.0)	276 (19–321)		2.1 (<0.5–6.0)	358 (35–807)	0.279 (0.124–2.868)	3,940 (1,352–9,635)	7.6 (2.3–9.8)
C	200	1.0 (0.5–1.0)	995 (712–1,087)		11.6 (10.3–14.3)	1,695 (1,567–2,318)	0.118 (0.086–0.128)	2,152 (1,334–3,499)	12.6 (10.7–19.0)
D	400	1.5 (1.5–4.0)	1,404 (1,343–3,337)		44.6 (27.5–54.0)	6,716 (2,984–7,049)	0.060 (0.057–0.134)	918 (774–2,612)	11.2 (9.0–13.5)
Multiple-dose (q12h) fasted cohorts									
F	100	1.7 (1.0–2.2)	461 (208–710)	6.8 (3.2–16.8)	12 (5–23)	937 (370–1,205)	0.115 (0.081–0.293)	2,663 (1,677–5,376)	15.9 (10.3–16.7)
G	200	1.8 (1.4–2.2)	793 (442–1,526)	31.0 (15.6–52.0)	33 (14–61)	1,590 (776–2,842)	0.126 (0.060–0.258)	1,923 (987–5,017)	11.2 (10.7–14.2)
I	400	1.8 (1.2–2.3)	2,305 (1,944–3,069)	122.0 (97.2–170.5)	137 (115–208)	7,662 (6,575–8,479)	0.052 (0.043–0.061)	966 (661–1,032)	12.0 (10.9–13.1)

[a] Predose C occurs 72 h following the initial dose and immediately prior to the 72-h (seventh) PK dose.
[b] For multiple-dose cohorts, C_{12} occurs 84 h following the initial dose and 12 h following the 72-h (seventh) PK dose.
[c] $AUC_{0-\infty}$ applies to single-dose cohorts; AUC_{0-12} applies to multiple-dose cohorts and covers the period 72 to 84 h following the initial dose and 0 to12 h following the 72-h (seventh) PK dose.

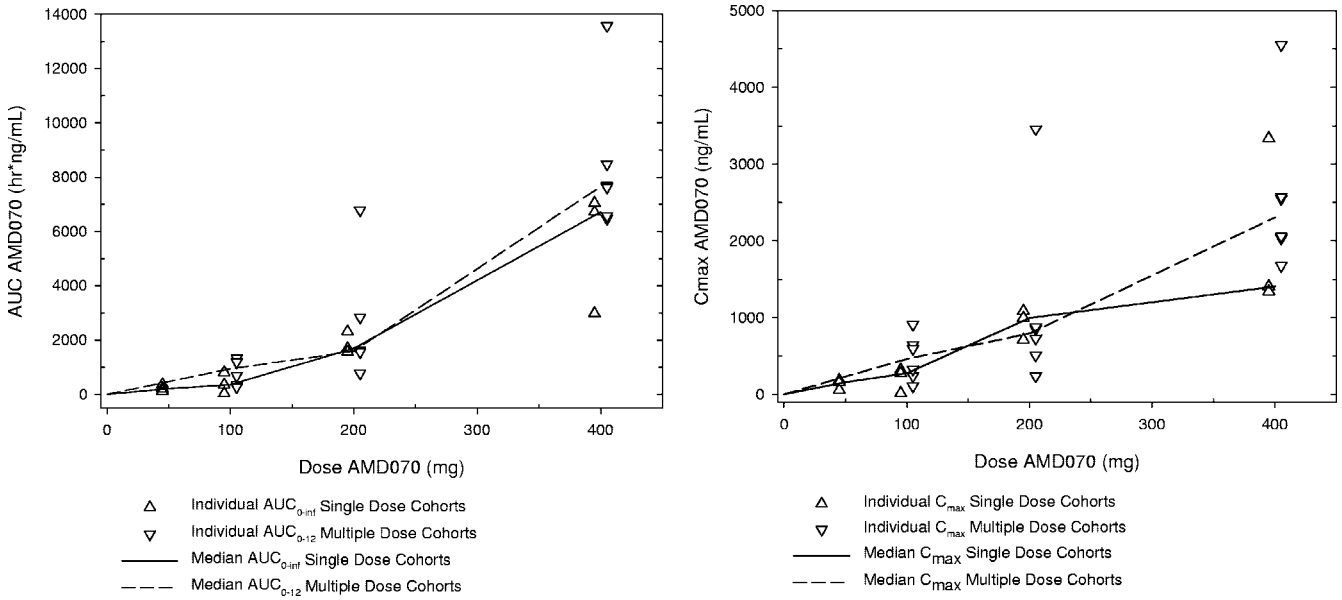

FIG. 2. Individual $AUC_{0-\infty}$ (left) and C_{max} (right) values versus dose for single (triangles)- and multiple (inverted triangles)-dose cohorts. Lines are drawn through dose cohort medians and origins.

unit-hours), or 6.2% below the expected value of 24 unit-hours ($P = 0.02$); the AUC_{0-12} was not significantly different from reference values ($P = 0.5$) for the multiple-dose cohorts. As the dose increased in the multiple-dose cohorts, there was an associated decline in the magnitude of increase seen in CD4 cell counts following dosing, measured as the C_{max} ($P = 0.03$), C_{24} ($P = 0.03$), AUC_{0-12} ($P = 0.05$), and AUC_{0-24} ($P = 0.06$), unlike the dose-related increases in total leukocytes.

Plotting either WBC count (cells/μl) or WBC ratio (WBC_{peak}/$WBC_{baseline}$) versus the log AMD070 concentration for all available concentrations for each subject showed a sigmoid concentration-response relationship for each subject except those in the lowest-dose (50 mg) cohort (data not shown). Pooling data from all subjects ($n = 232$), the WBC ratio versus AMD070 concentration (from all sampling times) best fit a sigmoid E_{max} model, with an estimated E_{max} of a 2.03-fold increase over baseline (95% confidence interval, 1.95- to 2.11-fold) and an EC_{50} of 39 ng/ml (95% confidence interval, 28 to 50 ng/ml) (Fig. 4). If different drug exposure parameters (dose, C_{max}, and AUC) were fit to the maximum WBC ratio for each subject ($n = 36$), using the simple E_{max} model, then C_{max} provided the best fit, with an E_{max} of 2.31 times the baseline for the maximum WBC ratio (95% confidence interval, 2.08 to 2.53 times the baseline) and an EC_{50} of 732 ng/ml (95% confidence interval, 205 to 1,260 ng/ml), although all parameters provided statistically significant parameter estimates for E_{max} and EC_{50}. Fitting either the pooled data ($n = 232$) or the

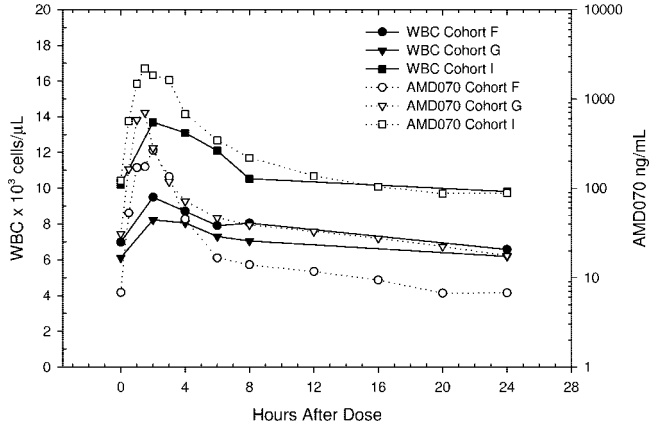

FIG. 3. WBC count versus time (closed symbols) overlaid with AMD070 concentration versus time (open symbols). Medians for each multiple-dose cohort are shown, using the same symbol for each cohort.

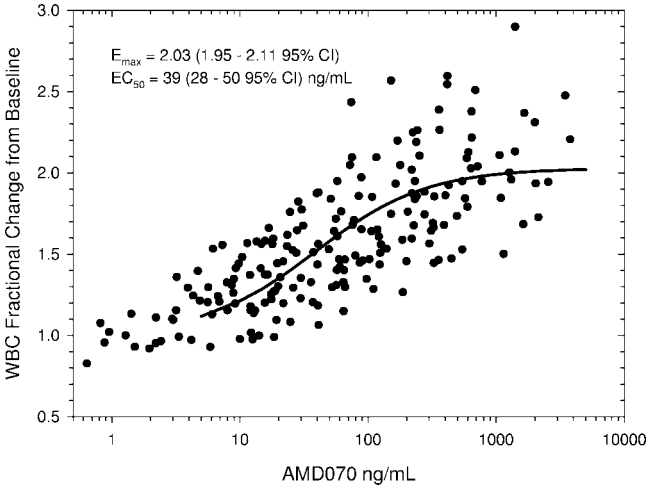

FIG. 4. Fractional change in WBC count relative to baseline versus AMD070 concentration. Data for all subjects and all time points were pooled ($n = 232$). The curve was predicted fit from the E_{max} model, including terms for E_{max} and EC_{50}.

individual data ($n = 36$) to a more complex E_{max} model with a sigmoidicity (Hill) coefficient did not improve the model fit.

DISCUSSION

This first-in-human study provides the safety, PK, and pharmacodynamic data for a new CXCR4 antagonist, AMD070, administered to healthy volunteers in single- and multiple-dose regimens. The drug was well tolerated by study participants, with no missed doses and no withdrawals from the study because of adverse effects. All subject complaints were mild in severity, with the exception of several moderate (defined as requiring analgesia) headaches and one subject with moderate diarrhea. All side effects were reversible, and there was no relationship to dose. The most common symptoms were headache, various gastrointestinal complaints, and vague neurologic symptoms. Electrocardiogram-confirmed sinus tachycardia was associated with symptoms of lightheadedness or palpitations in two subjects and was asymptomatic in five others.

The most frequently noted laboratory abnormality was CPK elevation at the 2-week follow-up visit. Several of the volunteers had baseline screening CPK levels above the normal range which normalized during the inpatient portion of the study while the drug was being administered. It was only after participants had been back to their regular routines for 2 weeks that the CPK values were increased. Given that the CPK level was not rising during AMD070 administration and the 2-week delay between the last dose and the follow-up labs, we felt that the CPK elevations were probably not due to AMD070. A few subjects also had bilirubin ($n = 2$) or lipase ($n = 4$) elevation, which was possibly related to AMD070 dosing. The absence of a concurrent placebo control limits our ability to determine how likely these laboratory signs or clinical symptoms were to be caused by the study drug.

AMD070 was orally bioavailable. AMD070 was slowly eliminated, with a terminal elimination $t_{1/2}$ of 11 to 16 h for the multiple-dose cohorts. The CL_T/F of AMD070 is primarily due to metabolism, as CL_R/F accounted for only 1% of CL_T/F. There was consistent evidence of mixed-order kinetics in the dose range studied, including disproportionately high fourfold C_{12} and fivefold AUC increases with only a doubling of the dose, from 200 to 400 mg q12h, in the multiple-dose cohorts; dose-dependent decreases in CL_T/F and V/F (though these changes might represent increases in bioavailability); a failure to achieve steady state after the passage of 4.5 to 6 $t_{1/2}$ (seven q12h doses) in the multiple-dose regimens; and nearly threefold increases in the AUC and C_{12} relative to comparable single-dose values, which is greater than expected given the estimated terminal $t_{1/2}$ of 11 to 16 h. Food resulted in significant decreases in both C_{max} and AUC_{0-24}, with median decreases of 70% and 56%, respectively, and nearly a doubling in time to peak concentration.

We expected the leukocytosis in this study based on our previous experience with AMD3100, a previous CXCR4 receptor antagonist (4, 5, 7, 9). We postulate that AMD3100 and AMD070 inhibit CXCR4-dependent interactions between bone marrow stromal cells and mature leukocytes of many lineages as well as $CD34^+$ stem cells, thus allowing release of these cells into the circulation (2, 9). The IQR of AMD070 concentrations 12 h after dosing for subjects in the two lower-dose multiple-dose cohorts (100 and 200 mg q12h) agreed with the EC_{50} for leukocytosis predicted by our pharmacodynamic model. Although the q12h doses clearly achieved concentrations in the biologically active range, the pharmacodynamic model of leukocytosis suggests that the concentrations associated with a maximum or plateau effect were reached only by the peak C_{max} concentrations of AMD070 achieved in this study.

Assuming that leukocytosis is a surrogate marker for inhibition of CXCR4, one might reasonably expect CXCR4-mediated inhibition of HIV infection in the range of doses studied here. Consistent with this expectation, the AMD070 concentrations 12 h after dosing in all 400-mg q12h cohort subjects were several times higher than the in vitro antiviral EC_{90} (adjusted for protein binding) for MT-4 cells. It is also possible that the pharmacodynamics, specifically CXCR4 receptor-ligand kinetic interactions, may be quite different for the leukocyte-bone marrow stromal cell interaction than for the HIV-CD4 cell interaction. For AMD3100, the doses that were associated with maximal WBC elevation were also associated with an antiviral effect, but there are insufficient data to determine if that antiviral effect was maximal, since only one subject was unequivocally evaluable for a CXCR4 effect by virtue of having pure X4 virus (4, 5). Similarly, there are insufficient data to compare the relative potency of AMD070 for the leukocytosis effect with that for the antiviral effect. Once a linkage between these relative potencies can be established in a clinical study, it may strengthen the argument for using leukocytosis as a relevant marker of an antiviral effect.

Another observation of note is the contrast in patterns between the overall leukocytosis (WBC and subsets, including CD34) and the CD4 cell changes seen after dosing. Unlike the dose-related leukocytosis, CD4 cell count elevations that occurred in the first 2 hours after dosing decreased in magnitude with an increasing dose and duration of AMD070 treatment. The CD4 cell counts 24 h after dosing actually declined by 20%, in a dose-dependent fashion; this decrease would only be relevant with cessation of dosing or with longer dosing intervals than the 12-h dosing interval we used. There were no statistically significant differences in the time-averaged CD4 cell counts (AUC_{0-12}) over the 12-h dosing interval used in the study. These CD4 AUC estimates, however, may not be relevant, as they do not account for the diurnal variation in CD4 cell counts seen in healthy subjects, which is typically blunted in persons with HIV infection (10).

In summary, AMD070 was a safe, well-tolerated drug when administered to healthy volunteers. The PK profile suggests oral bioavailability, a significant reduction in drug exposure in the presence of food, and mixed-order pharmacokinetics in the dose range studied. Based on leukocytosis as a surrogate for CXCR4 inhibition, the doses used in this phase I study were biologically active in vivo and demonstrated a concentration-related response. Pharmacodynamic modeling based on the leukocytosis effect of AMD070 suggests that higher doses might be required to sustain a maximal effect throughout the dosing interval. These studies support further development of AMD070 as an oral CXCR4 antagonist for the treatment of HIV infection.

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ERRATUM

Multiple-Dose Escalation Study of the Safety, Pharmacokinetics, and Biologic Activity of Oral AMD070, a Selective CXCR4 Receptor Inhibitor, in Human Subjects

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Volume 51, no. 7, p. 2351–2358, 2007. Page 2354, column 2, line 28: "0.114×10^{-2} liter/h (0.075×10^{-2} to 1.36×10^{-2})" should read "1.14 liter/h (0.75 to 1.36)."

Page 2355, Table 3: The values in the "CL/*F* (liter/h)" column should be, from top to bottom, 231 (135–386), 279 (124–2,868), 118 (86–128), 60 (57–134), 115 (81–293), 126 (60–258), and 52 (43–61).