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A phase I trial of the pharmacokinetics, toxicity, and activity of KNI-272, an inhibitor of HIV-1 protease, in patients with AIDS or symptomatic HIV infection

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Abstract

The pharmacokinetics, toxicity, and activity of KNI-272, a transition state inhibitor of HIV-1 protease, was assessed in a phase I trial. After an initial phase in which the pharmacokinetics were assessed, 37 patients with AIDS or symptomatic HIV infection and 100–400 CD4 cells/mm^3 were entered in an escalating dose study. KNI-272 was administered four times daily for up to 12 weeks. Oral bioavailability ranged from 22 to 55% and was not appreciably different in the fasting and post-prandial state. The dose limiting toxicity was hepatic transaminase elevation; this could be reduced by escalating the dose over 4 weeks. When administered this way, the maximum tolerated oral dose was 40 mg/kg per day. At the highest two tolerated doses (26.4 and 40 mg/kg per day), there was some evidence of an anti-HIV effect with median decreases of 0.2–0.3 $\log_{10}$ copies/ml plasma HIV RNA; these decreases persisted through 7–8 weeks of treatment. There was an upward trend in the CD4 count at the 40 mg/kg per day dose but not at other doses. Additional studies focused on approaches to improve the therapeutic index of KNI-272 may be warranted.

Keywords: KNI-272; HIV-1 protease; AIDS; HIV; Protease inhibitor

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1. Introduction

Several inhibitors of the HIV-1 protease have recently been introduced into clinical use and their success, particularly in combination therapy with inhibitors of HIV-1 reverse transcriptase, has enabled greater suppression of HIV replication in patients than had been attainable with previous regimens (Dreyer et al., 1989; Erickson et al., 1990; Kempf et al., 1990; McQuade et al., 1990; Roberts et al., 1990; Danner et al., 1995; Markowitz et al., 1995; Schapiro et al., 1996; Stein et al., 1996; Deeks et al., 1997). However, resistance to these agents can develop relatively rapidly, and many patients develop unacceptable side effects (Gulnik et al., 1995; Ridky and Leis, 1995; Tisdale et al., 1995; Molla et al., 1996; Patick et al., 1996; Stein et al., 1996; Ginsburg et al., 1997; Tisdale et al., 1997; Carr et al., 1998). For these reasons, it is important to design and develop new members of this class of drugs.

KNI-272 (Fig. 1) is a transition state mimetic tripeptide inhibitor of the HIV-1 protease that contains a unique unnatural amino acid, allophenylnorstatine (Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) with a hydroxymethylcarbonyl isostere as the active moiety (Mimoto et al., 1992; Kageyama et al., 1993). This drug has activity against a wide spectrum of HIV strains with 50% inhibitory concentrations (IC_{50}) ranging from 0.08 to 0.1 μM in vitro (Kageyama et al., 1993). It is active in both resting and activated lymphocytes (Chokekijchai et al., 1995). Moreover, it was recently demonstrated that HIV viral particles produced in the presence of KNI-272 do not mature to form infectious particles after their release from cells if the drug is withdrawn from the culture media in which the particles are suspended (Humphrey et al., 1997). KNI-272 was found to be well absorbed by dogs when taken orally with bioavailabilities of 40–60% (Kiriyama et al., 1993, 1994). Preclinical toxicity studies indicated that KNI-272 could be orally administered to beagle dogs at doses that produced plasma concentrations above the IC_{50} of HIV-1 without substantial toxicities.

With this background, we initiated a phase I trial of an oral formulation of KNI-272 to evaluate the toxicity and pharmacokinetics of the drug and to ascertain preliminary information on its activity against HIV-1.

2. Materials and methods

2.1. Patients

The clinical trial had a two-stage design. In the first stage, patients were administered three doses of KNI-272 for pharmacokinetic analysis and short-term toxicity assessment. In the second stage, patients were administered KNI-272 for up to 12 weeks to assess the drug's pharmacokinetic behavior, toxicity and anti-HIV activity. Six patients (four men, two women, age range: 33–64 years) were enrolled onto the first stage, and 37 patients (34 men, three women; age range: 27–62 years) were enrolled onto the second stage (Table 1). All patients were treated at the Warren G. Magnuson Clinical Center of the National Institutes of Health in Bethesda, Maryland. The protocol for this study was reviewed by the National Cancer Institute Institutional Review Board, and human experimentation guidelines of the US Department of Health and Human Services were followed. All patients participating on the study gave informed consent.

Men and non-pregnant women who were infected with HIV and who were at least 18 years of age were eligible. Patients were required to have CD4 counts between 100 and 400 cells/mm^3 at the time of screening for the protocol and either AIDS, as defined by the 1993 AIDS surveillance

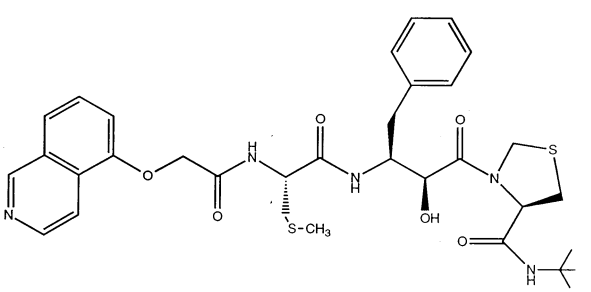

Fig. 1. Chemical structure of KNI-272.

Table 1
Characteristics of patients studied on escalating dose phase[a]

	Daily dose of KNI-272 (mg/kg)					
	Non-escalating dose schedule			Escalating dose schedule		
	8.0	16.0	26.4	26.4	40.0	56.0
No. of patients	3	8	5	6	7	8
Mean age, years (range)	36.6 (33–41)	40.6 (33–53)	42.2 (34–50)	39.8 (31–49)	42.8(30–62)	40 (27–50)
Gender (men/women)	3/0	8/0	5/0	5/1	6/1	7/1
No. of patients with AIDS Risk factor	2	4	4	5	5	5
Sex with men	2	6	4	3	2	7
Sex with men and IVDU	0	1	1	1	2	0
Sex with men and BT	0	1	0	0	2	0
Heterosexual contact and BT	1	0	0	0	0	0
Heterosexual contact	0	0	0	2	1	1

[a] IVDU, intravenous drug user; BT, blood transfusion.

case definition (Anonymous, 1994), or symptomatic HIV infection. For the purposes of this study, symptomatic HIV infection was defined as a history of or active oral candidiasis, oral hairy leukoplakia, herpes zoster after infection with HIV-1, recurrent seborrheic dermatitis, pruritic folliculitis, weight loss of greater than 4.5 kg or greater than 10% of body weight not caused by dieting, unexplained intermittent diarrhea, night sweats, cognitive impairment or fatigue interfering with activity. At the time of entry (week 0), the median CD4 count of the patients entered onto the second stage was 183 cells/mm^3 (range 77–483 cells/mm^3).

Patients were excluded from the study if they had a hemoglobin level of less than 9.0 g/dl, an absolute neutrophil count of less than 1000/mm^3, or a platelet count of less than 75 000/mm^3. Patients were also excluded if they had substantial renal, hepatic, cardiac, or neurologic abnormalities; a history of pancreatitis; severe malabsorption; active opportunistic infections; or tumors likely to require cytotoxic anti-tumor therapy within 6 months after entering the study. Eligible patients could not have previously received other HIV-1 protease inhibitors nor could they have received suramin, ribavirin, foscarnet, ganciclovir, cytotoxic antineoplastic agents, steroids, interferon, immunomodulating agents or any investigational drugs within the preceding 3–4 months prior to study entry. Patients had to refrain from using other antiretroviral drugs for the 3 weeks prior to study entry and while receiving KNI-272. Of the 37 patients entered onto the second stage, 35 had received prior antiretroviral therapy and 32 had received more than 6 months of such therapy.

2.2. Treatment regimen

KNI-272 was manufactured by Japan Energy Company. The drug product was formulated and supplied by the Developmental Therapeutics Program of the National Cancer Institute (NCI). Initially, the drug was supplied as a two-container system consisting of a vial containing KNI-272 150 mg as a sterile lyophilized powder with 3000 mg of hydroxypropyl-β-cyclodextrin (HPCD) and a bottle containing 50 ml of 5% citric acid solution for reconstituting the powder. The drug product was reconstituted to a concentration of 3 mg/ml of KNI-272 and (when intended for oral use) flavored with cherry syrup before administration. Later in the trial, KNI-272 was supplied as gelatin capsules containing 50, 100 or 150 mg of KNI-272 and anhydrous citric acid.

The first stage of the trial was a three-dose pharmacokinetic study involving six patients. For each of two dose levels (2 and 4 mg/kg per dose), each of three patients received a single intravenous (IV) dose of KNI-272 on day 1, a single oral dose in the fasting state on day 2 and a single oral dose with food on day 3. Patient were entered onto this stage between March and June, 1994.

Once dosing on the first stage was completed, patient enrollment was initiated on the second stage. Patient enrollment on this stage started in July, 1994 and ended in September of 1996. Patients who had successfully completed the first stage without toxicity were eligible for rentry onto this stage, in which three to eight patients were treated at each dose level for several weeks. Patients received an intravenous dose on day 1 and single oral doses on days 2 and 3 to assess pharmacokinetics. They then received drug by mouth every 6 h. Initially the liquid formulation in cherry syrup was utilized for oral dosing, but during the 4 mg/kg dosing, this was switched to the capsule formulation. The patients were first treated for up to 4 weeks, but the protocol was subsequently amended to allow dosing of up to 12 weeks when supportive animal toxicity data became available. Three dose levels (8, 16 and 26.4 mg/kg per day) were initially evaluated. As described below, elevated hepatic transaminase levels proved to be the dose-limiting toxicity of KNI-272 when administered in this manner. In an attempt to circumvent this problem, an alternate dosing schedule was then evaluated in which the patients received KNI-272 in increasing doses over four weeks until the target dose was reached. On this schedule, patients received 25% of the target dose during the first week of therapy, 50% during the second week, 75% during the third week, and 100% of the target dose thereafter. Three dose levels (26.4, 40 and 56 mg/kg per day) were evaluated using this strategy with each patient receiving up to 12 weeks of KNI-272 (9 weeks at the target dose).

2.3. Patient evaluation

Patients were closely monitored for toxicity and for other clinical and laboratory changes while on the study. Assessment at the time of enrollment included a complete physical examination, chest radiograph, electrocardiogram, complete blood cell count, measurements of serum chemistries, urinalysis, and, if applicable, a pregnancy test. Patients also had baseline measurements of their lymphocyte subsets and plasma HIV genome by quantitative RNA PCR (Roche Amplicor HIV-1 monitor assay kits, Roche Diagnostic Systems, Branchburg, NJ). The baseline (week 0) evaluation of lymphocyte subsets (by fluorescent activated cell sorter analysis) was obtained by averaging the values obtained from 2 to 3 measurements made at least 24 h apart within 2 weeks of the first dose. In those patients in which two values for HIV RNA-PCR were available prior to treatment, the baseline value was considered as the geometric mean of the values. Two patients for whom a baseline RNA-PCR value was not obtained prior to treatment were censored in regard to analysis of this parameter.

Patients were evaluated weekly for the first 4 weeks and every 2 weeks thereafter. Chemistry and hematology evaluations were done at each assessment. In addition, lymphocyte determinations and measurements of quantitative RNA PCR were performed at least every 2 weeks. For the purposes of this study, toxicities were graded using the NCI Common Toxicity Criteria (Wittes, 1991) with exception of hepatic transaminases for which the cut-offs were modified as follows: 76–125 IU/ml was defined as grade 1 toxicity; 126–250 IU/ml was defined as grade 2 toxicity; 251–500 IU/ml was defined grade 3 toxicity; and greater than 500 IU/ml was defined as grade 4 toxicity. These modifications were made to reflect the higher baseline values generally found in patients with symptomatic or advanced HIV infection.

2.4. Pharmacokinetics

Pharmacokinetic studies were performed after each of the three doses on all patients in the first stage of the study. For patients enrolled in stage 2, pharmacokinetic studies were performed on at least three patients from each dose level after an initial single intravenous dose on day 1, a single oral dose in the fasting state on day 2, and a

Table 2
Estimates of steady-state pharmacokinetic parameters after KNI-272 administration[a]

Dosage (mg/kg per dose)	Route/ dose form[b]	n	C_{Max} (μM)	T_{Max} (h)	AUC (μM/h)	$t_{1/2}$ (γ) (h)	F (%)
2	Intravenous	6	5.48 ± 0.14	0.67 ± 0.11	5.16 ± 0.25	0.41 ± 0.04	NA
	Oral cherry syrup (fasting)	3	1.05 ± 0.11	1.11 ± 0.11	1.12 ± 0.19	NA	20.33 ± 3.44
	Oral cherry syrup (post-prandial)	3	0.61 ± 0.25	1.17 ± 0.67	0.85 ± 0.31	NA	15.72 ± 5.96
	Oral capsule (fasting)	3	2.42 ± 0.68	0.67 ± 0.00	1.84 ± 0.46	NA	33.99 ± 9.73
4	Intravenous	9	9.83 ± 0.47	0.50 ± 0.00	9.59 ± 0.81	0.52 ± 0.04	NA
	Oral cherry syrup (fasting)	3	1.62 ± 0.41	1.00 ± 0.19	1.95 ± 0.38	NA	20.50 ± 2.24
	Oral capsule (fasting)	6	2.72 ± 0.41	0.78 ± 0.16	2.79 ± 0.40	NA	29.56 ± 3.50
	Oral capsule (post-prandial)	5	1.64 ± 0.60	0.87 ± 0.08	2.10 ± 0.48	NA	22.06 ± 2.99
6.6	Intravenous	2	15.30	0.50	14.39	0.39	NA
	Oral capsule (fasting)	2	3.82	1.00	4.85	NA	33.48
	Oral capsule (post-prandial)	2	3.53	2.00	5.16	NA	36.40
10	Oral capsule (fasting)	3	7.48 ± 2.30	1.22 ± 0.40	9.12 ± 1.65	NA	54.97 ± 19.78
14	Oral capsule (fasting)	3	5.51 ± 1.17	1.11 ± 0.29	9.70 ± 4.04	NA	31.13 ± 12.98

[a] NA, not applicable.
[b] Data presented are from thepharmacokinetics performed at the beginning of the study except for the 10 mg/kg per dose. Results from the latter represent values obtained after 4 weeks (i.e. after the 3 weeks of the escalating dosing regimen followed by 1 week of full dose KNI-272). $t_{1/2}$ (γ) is only shown for the intravenous doses because the values after oral dosing are not exclusive of the effects of late absorption.

single oral dose (with food) on day 3. For patients enrolled in the initial phase of stage 2 only (those patients who, when started on multiple daily oral doses, were started immediately on the full target dose of KNI-272), pharmacokinetic studies were also done on day 4 (the first day of multiple oral doses) and at the end of weeks 2 and 4. For patients enrolled in the arm of stage 2 in which the dose was increased to the target dose over 4 weeks, pharmacokinetic studies were done after an observed oral dose at the end of weeks 1, 4, 8 and 12. It should be noted, however, that during these pharmacokinetic studies, the time 0 concentrations represented the trough levels after the previous unobserved dose. In all cases, the plasma concentration of KNI-272 was determined by high-performance liquid chromatography as described (Kiriyama et al., 1994).

2.5. Statistical analysis

The change in CD4 counts and in HIV serum RNA levels at various time points compared with baseline was evaluated by Wilcoxon signed-rank test; HIV RNA levels were logarithmically transformed prior to analysis. Comparisons of the bioavailability of the liquid and capsule preparations of KNI-272 were evaluated by the paired t-test.

3. Results

3.1. Pharmacokinetics

The estimates of steady-state pharmacokinetic parameters after KNI-272 administration obtained during the first and second stages of the study are presented in Table 2. For both the 2 and 4 mg/kg dose levels, the oral capsule provided a slightly higher peak plasma concentration and oral bioavailability (F) than the oral liquid formulation furnished with cherry syrup although this difference was not statistically significant ($P >$

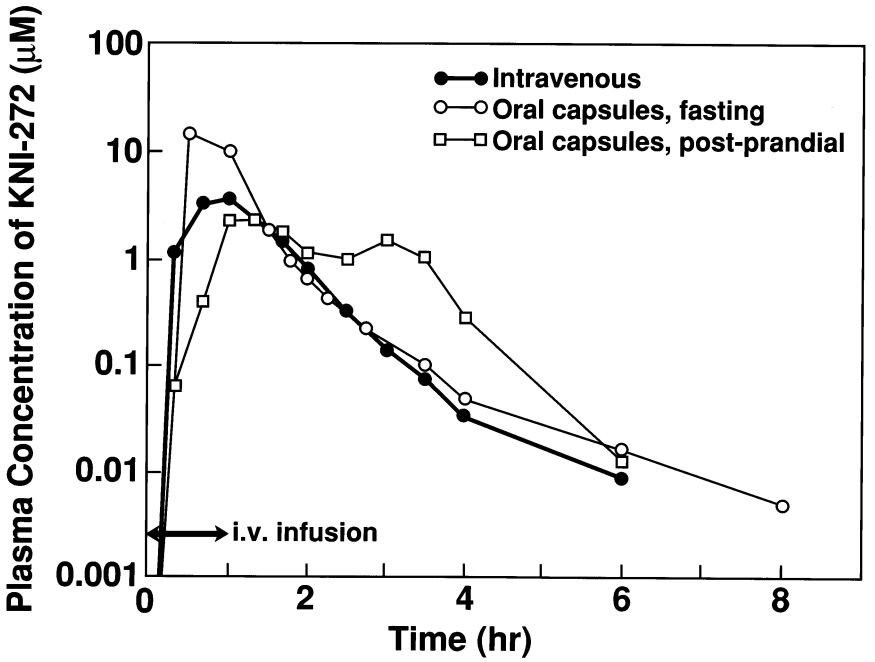

Fig. 2. Plasma concentrations of KNI-272 after intravenous and oral administration of capsules at a dose of 6.6 mg/kg in patients with HIV infection.

0.05). Bioavailability of the capsule formulation was not appreciably different in the fasting and post-prandial state and overall ranged from approximately 22% to 55%. In considering the level of drug measured in the plasma, it is worth noting that the IC_{50} of KNI- 272 against isolates of HIV-1 have been found to range between 0.004 and 0.1 μM under usual culture conditions (Kageyama et al., 1993, 1994). The peak plasma concentrations (C_{Max}) achieved during administration of KNI-272 at dose levels greater than or equal to the 6.6 mg/kg per dose are substantially higher than those concentrations (Table 2 and Fig. 2). Except at the highest dose tested (14 mg/kg) trough serum levels were approximately 0.1 μM, but they dipped below that level at lower doses (Fig. 2 and results not shown). Representative curves (averaged from the values obtained from two patients) of plasma concentrations of KNI-272 following intravenous and oral administration at the 6.6 mg/kg dose level of KNI-272 are presented in Fig. 2. The mean half-lives ($T_{1/2}\gamma$) of the drug administered intravenously were 0.41 ± 0.04 h after the 2 mg/kg doses, 0.52 ± 0.04 h after the 4 mg/kg doses, and 0.39 h after the 6.6 mg/kg doses. When increasing fractions of the full target dose were administered over three weeks in the scale-up dosing regimen, the area under the plasma concentration–time curve (AUC) was linearly related to the dose (data not shown), suggesting that drug metabolism was not up-regulated by this dosing strategy.

3.2. Clinical and laboratory toxicities

After completing three days of single dose administration with pharmacokinetic sampling, patients enrolled early in the second stage received drug four times daily for 4–12 weeks. The dose-limiting toxicity was observed to be hepatic transaminase elevation and the toxic dose was 6.6 mg/kg per dose (26.4 mg/kg per day; Tables 3 and 4). At this dose, grade 3 elevations in hepatic transaminases developed in two of five patients tested. The transaminase elevations started within

Table 3
Clinical toxicities during KNI-272 therapy[a]

Dose and toxicity	Grade 2	Grade 3	Grade 4
8 mg/kg per day ($n = 3$)	0	0	0
16 mg/kg per day ($n = 8$)			
Diarrhea	1	0	0
Throat pain	1	0	0
26.4 mg/kg per day ($n = 5$)			
Diarrhea	1	0	0
26.4 mg/kg per day, escalating dose ($n = 6$)			
Diarrhea	1	0	0
40 mg/kg per day, escalating dose ($n = 7$)			
Abdominal pain	2	0	0
Diarrhea	1	0	0
Nausea	1	0	0
56 mg/kg per day, escalating dose ($n = 8$)			
Abdominal pain	1	0	0
Diarrhea	1	0	0

[a] As defined by the Common Toxicity Criteria, Clinical Trials Evaluation Program, National Cancer Institute (Wittes, 1991).

the first week of therapy and promptly declined (within 24 h) after discontinuation of KNI-272. The maximum tolerated dose (MTD) on this schedule was thus defined as 4 mg/kg per dose (16 mg/kg per day).

It was noteworthy that in two patients who experienced grade 2 elevations in hepatic trasaminases, enzyme levels returned to normal even though the drug was continued. These results, along with the rapid decline in transaminases when KNI-272 was stopped, suggested that tolerance may develop to KNI-272. Pharmacokinetic studies in patients after several weeks on drug did not point to changes in the plasma concentration-time AUC as a basis for this tolerance. However, even if this did not appear to be a change in the pharmacokinetic profile, we hypothesized that gradually increasing the dose of drug might enable higher doses to be administered without unacceptable toxicity being attained. Therefore, additional patients were entered on a scale-up dosing regimen in which a given patient's dosage was incrementally increased over 4 weeks (Section 2).

With this new scale-up dosing regimen, we were able to treat patients with dosage levels of 26.4 and 40 mg/kg per day without observing substantial hepatotoxicity. Only grade 2 hepatic transaminase elevations were noted at these dosages and they resolved spontaneously without discontinuation of KNI-272. Using this strategy, dose limiting toxicity was observed at a level of 14 mg/kg per dose (56 mg/kg per day); the dose-limiting toxicity at this level was again reversible hepatic transaminase elevation. Therefore, the MTD on this schedule was defined as 40 mg/kg per day (10 mg/kg per dose).

The clinical and laboratory toxicities (above grade 2) that were encountered during the second stage (toxicity assessment) of the study are summarized in Tables 3 and 4, respectively. The laboratory toxicities that are listed in Table 4 include only those that were observed after treatment with KNI-272 was initiated. That is, individuals who demonstrated grade 2 laboratory values at baseline which did not change substantially after treatment was initiated with KNI-272 are not listed on the table. Eleven of 37 individuals who received extended dosing of KNI-272 complained of mild (grade 1) esophageal burning following ingestion of KNI-272 pills (at all dosages).

Table 4
Laboratory toxicities during KNI-272 therapy[a]

Dosage and toxicity	Grade 2	Grade 3	Grade 4
8 mg/kg per day ($n = 3$)			
Hyperglycemia	1	0	0
16 mg/kg per day ($n = 8$)			
Increased amylase	0	1	0
Decreased neutrophil count	1	1	0
Hyperglycemia	1	0	0
Increased hepatic transaminases	1	0	0
26.4 mg/kg per day ($n = 5$)			
Increased hepatic transaminases	1	2	0
Decreased neutrophil count	2	1[b]	0
Decreased white blood cell count	1	0	0
26.4 mg/kg per day, escalating dose ($n = 6$)			
Increased alkaline phosphatase	0	1	0
Increased hepatic transaminases	2	0	0
Hypoglycemia	1	0	0
40 mg/kg per day, escalating dose ($n = 7$)			
Decreased white blood cell count[e]	1	1	0
Decreased neutrophil count	1	1	0
Increased amylase	1	0	0
Increased hepatic transaminases	3	0	0
56 mg/kg per day, escalating dose ($n = 8$)			
Increased hepatic transaminases	2	2[c]	1
Increased amylase	0	1[d]	0
Decreased white blood cell count	1	0	0
Decreased neutrophil count	2	0	0

[a] As defined by the Common Toxicity Criteria, Clinical Trials Evaluation Program, National Cancer Institute (Wittes, 1991).
[b] Patient had grade 2 neutropenia prior to initiation of KNI-272 therapy.
[c] One patient took approximately 1950–2600 mg acetominophen daily for 3 days for an upper respiratory infection.
[d] Patient had grade 2 increase in amylase prior to initiation of KNI-272 therapy.

3.3. Anti-HIV activity

Changes in the plasma viral RNA measurements during KNI-272 administration are shown in Fig. 3. There was no decrease in plasma viral RNA measurements in patients receiving less than 26.4 mg/kg per day of KNI-272. There was, however, a trend downward in patients receiving 26.4 mg/kg per day of drug, and this was more pronounced on the 40 mg/kg per day dosage level. At the latter dosage level, the plasma viral RNA at weeks 6 and 8 decreased from baseline by 0.2 $\log_{10}$ and 0.3 $\log_{10}$, respectively. Taken separately, none of these changes were statistically significant although the small sample size at each of the doses limited the ability to detect such changes. However, when data from the last three dosage level cohorts (26.4 mg/kg per day without escalating doses, 26.4 mg/kg per day with escalating doses, and 40 mg/kg per day with escalating doses) were pooled, a statistically significant decrease in plasma viral RNA was noted after 3–4 weeks of full-dose therapy ($P = 0.049$ by Wilcoxon signed rank test). This decrease persisted through 7–8 weeks of treatment with a mean decline of approximately 0.19–0.22 $\log_{10}$ ($P < 0.05$ by Wilcoxon signed rank at 5–6 and 7–8 weeks, not adjusted for the number of tests) at each time point. Changes in RNA-PCR were not statistically significant at 9–10 weeks.

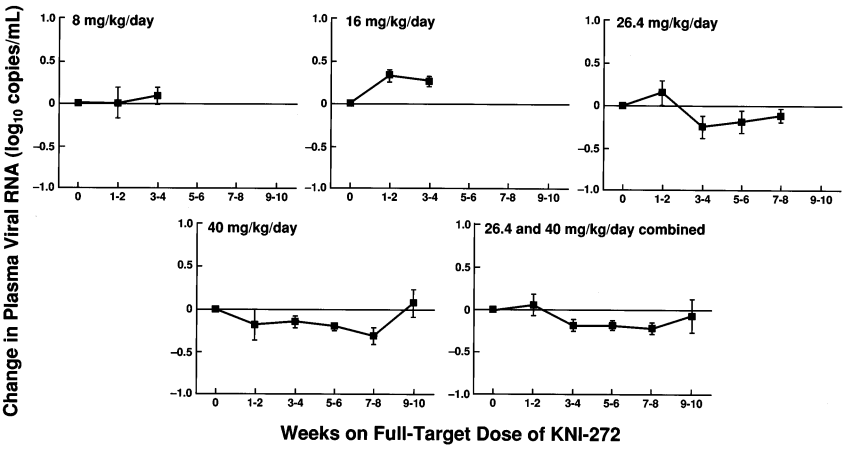

Fig. 3. Changes in viral load as assessd by plasma HIV RNA PCR during administration of KNI- 272. The 26.4 mg/kg per day graphs includes both the patients on the non-escalating and the escalating dose regimens. Results shown are the mean ± SEM. The number of patients still evaluable at week 8 were four for the 26.4 mg/kg per day dose, and five for the 40 mg/kg per day dose.

Changes in the absolute CD4 count during KNI-272 therapy are shown in Fig. 4. While there was a trend upward in CD4 counts in patients who received 40 mg/kg per day, this was not significant and there were no other trends in the CD4 counts in patients who received lower dosages. Also, patients who received 56 mg/kg per day did not demonstrate clear changes from baseline in either their CD4 counts or plasma HIV RNA (data not shown). In view of the large number of capsules that patients were required to take for each dose at this dosage level (15–20 capsules/dose), the failure to detect a significant anti-HIV effect at 56 mg/kg per day of KNI-272 might possibly be explained in part by poor patient compliance. Evidence in support of this theory comes from measurements of the mean pre-dose plasma concentrations of KNI-272 after 12 weeks of therapy which were substantially lower in patients who received 56 mg/kg per day as outpatients compared to those receiving 40 mg/kg per day of KNI-272 (data not shown). Also, two of the patients reported missing between three and five doses. Pharmacokinetic studies done at week 12 did not suggest either decreased absorption when taken in a fasting state or increased drug clearance as compared to earlier analyses (data not shown). In summary, the data suggest that slight anti-HIV activity is observed with KNI-272 (assessed by changes in the circulating viral RNA measurements) in the 26.4–40 mg/kg per day dosage range.

4. Discussion

The results of this study demonstrate that KNI-272 is well absorbed after oral ingestion and that plasma levels well above the IC_{50} are attainable at doses that are generally tolerated for a 12-week period of time. The dose-limiting toxicity of this drug was found to be hepatic transaminase elevations which could be somewhat mitigated by incremental dosage escalations to a target dosage over 4 weeks. Finally, the results of this study show that some suppression of HIV replication (as assessed by HIV RNA PCR) was attained at the highest tolerated dosages (26.4–40 mg/kg per day given in four divided doses).

Pharmacokinetic studies in the patients who received KNI-272 revealed that peak serum levels achieved during drug administration (approximately 3.5 to 7 μM) were considerably higher than the IC_{50} of KNI-272 determined in vitro against common clinical isolates of HIV-1 (Kageyama et al., 1993; Chokekijchai et al., 1995). One explanation for the discrepancy between in vitro efficacy and clinical activity is likely to be

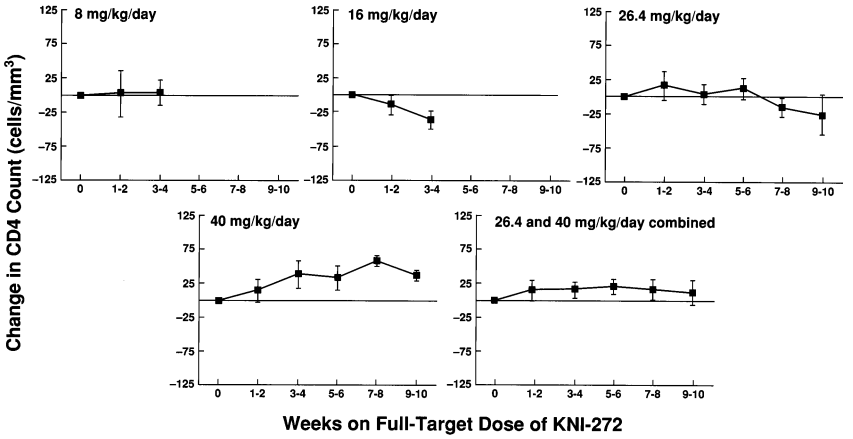

Fig. 4. Changes in absolute CD4 counts during administration of KNI-272. The 26.4 mg/kg per day graphs includes both the patients on the non-escalating and the escalating dose regimens. Results shown are the mean ± SEM.

the extensive KNI-272 binding to circulating plasma proteins. The IC_{50} of KNI-272 against HIV-1 isolates has been found to be increased by 25–100-fold in the presence of 80% fetal calf serum in vitro (Kageyama et al., 1994). More detailed studies of the protein binding of KNI-272 in human serum suggest that the drug is bound predominantly to α_1-acid glycoprotein. Extrapolating from these studies, it is estimated that KNI-272 is likely to be approximately 98–99% protein-bound in the circulating blood of patients receiving this agent (Kageyama et al., 1994). Thus, it is quite conceivable that even at the highest tolerated dose levels, therapeutic concentrations are attained only for brief periods following each dose. In this manner, KNI-272 appears to be similar to SC-52151, another protease inhibitor with extensive protein binding and less clinical activity than predicted from a comparison of in vitro activity and attained plasma levels (Fischl et al., 1997). The experience with these two drugs provides a cautionary note that the possible effects of binding to plasma proteins should be considered in the development of new protease inhibitors.

An alternative explanation for the discrepancy between in vitro efficacy and in vivo anti-HIV-1 activity may relate to the emergence of resistance in patients. When HIV-1 was passaged in the presence of increasing concentrations of KNI-272 in vitro, the protease-encoding gene acquired several mutations at codons 32, 33, 45, 53, 71, 84 and 89 that were together associated with a substantial decrease of binding to the protease (Yamada et al., 1996). However, none of these mutations were noted in the HIV-1 strains isolated from seven patients who received KNI-272 in a parallel study being carried out in children (Yamada et al., 1996; Mueller et al., 1998). Also, the patients on the present study did not have a substantial transient decrease in viral load during the first few weeks as one might expect with a highly active agent to which resistance then rapidly developed. For these reasons, it is unlikely that substantial resistance developed during the 12 weeks of this study.

In the present study, the maximum tolerated dose was successfully increased from 16 to 40 mg/kg per day using a strategy that involved administration of increasing fractions of the full target dose over 3 weeks. This strategy was based on observations from the non-escalated phase of the trial suggesting that tolerance to the hepatic toxicity produced by KNI-272 develops over several weeks. The mechanism for this tolerance is at this point unclear. Pharmacokinetic studies failed to find a change in the disposition of the drug during the first several weeks of therapy, indicating that tolerance was not simply the result of increased KNI-272 metabolism. It remains possible, however, that the disposition of a toxic

metabolite of KNI-272 is enhanced over several weeks of dosing. Investigation into this phenomenon may lead to strategies that increase the therapeutic index of KNI-272 and augment its clinical utility. At the same time, similar dosing strategies might be worth exploring for other agents that induce dose-limiting hepatic toxicity.

It is now recognized that resistance to protease inhibitors can develop quite rapidly, often within weeks of initiating therapy, and that there is substantial cross-resistance among these agents (Condra et al., 1995; Molla et al., 1996; Lorenzi et al., 1997). In addition, it has been shown that suppression of HIV replication with highly active regimens to the point where HIV is undetectable in the plasma by RNA PCR can substantially reduce the development of resistance to the drugs utilized (Condra et al., 1996). This information, plus an accumulation of data showing that the viral load in HIV-infected patients is predictive of their subsequent clinical course (Mellors et al., 1996; Hirsch et al., 1998), have led to a reconsideration of trial design for the initial development of protease inhibitors since the time that this study was initiated. By today's standards, the trial design used here (involving several weeks of monotherapy) would not be utilized with new protease inhibitors entering the clinic. Instead, alternate developmental strategies, for example in which the new agent is initially tested in normal volunteers, and is then tested with other agents, or is added to an existing anti-HIV drug regimen, are now being explored. Such approaches can also have drawbacks, however, and the optimal strategy for clinial anti-HIV drug development is an area of active discussion. A similar concern regarding the development of resistance would also apply to the strategy of escalating the dose of KNI-272 (or other protease inhibitors) over several weeks, although this could potentially be addressed by combination with other agents that could completely suppress HIV during this escalation period.

The ultimate role of KNI-272 in the fight against advancing HIV infection is not clearly established at this time. As a single agent, KNI-272 has a narrow therapeutic index and modest anti-HIV activity. However, like saquinavir, KNI-272 is metabolized by hepatic p450 enzymes (Schapiro et al., 1996). Thus, although KNI-272 has better bioavailability than saquinavir, administration of KNI-272 with an inhibitor of the p450 enzyme may still result in a more prolonged half-life and flattening of its pharmacokinetic profile (Lorenzi et al., 1997; Hsu et al., 1998). This may substantially increase the therapeutic index if toxicity is related to the peak levels of this drug. It is likewise possible that other strategies may be developed that reduce the toxicity associated with KNI-272 or that it will be found to have some utility in combination regimens. Future studies will be necessary to define the potential clinical role of this agent.

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