Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry

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T-20, a synthetic peptide corresponding to a region of the transmembrane subunit of the HIV-1 envelope protein, blocks cell fusion and viral entry at concentrations of less than 2 ng/ml *in vitro*. We administered intravenous T-20 (monotherapy) for 14 days to sixteen HIV-infected adults in four dose groups (3, 10, 30 and 100 mg twice daily). There were significant, dose-related declines in plasma HIV RNA in all subjects who received higher dose levels. All four subjects receiving 100 mg twice daily had a decline in plasma HIV RNA to less than 500 copies/ml, by bDNA assay. A sensitive RT–PCR assay (detection threshold 40 copies/ml) demonstrated that, although undetectable levels were not achieved in the 14-day dosing period, there was a 1.96 $\log_{10}$ median decline in plasma HIV RNA in these subjects. This study provides proof-of-concept that viral entry can be successfully blocked *in vivo*. Short-term administration of T-20 seems safe and provides potent inhibition of HIV replication comparable to anti-retroviral regimens approved at present.

The therapies now approved for human immunodeficiency virus type 1 (HIV-1) infection inhibit one of two viral-specific enzymes, reverse transcriptase or protease[1]. Although several combination regimens targeting these enzymes have considerable anti-retroviral activity[2–4], many patients are either intolerant of available agents[5,6] or develop virologic failure because of incomplete viral suppression and the emergence of drug-resistant virus strains[7,8]. Thus, development of new classes of anti-retroviral compounds with different mechanisms of action and toxicity profiles is an important goal.

The HIV-1 envelope glycoprotein consists of two noncovalently associated subunits, a surface glycoprotein (gp120; SU) and a transmembrane glycoprotein (gp41; TM). Portions of gp120 bind to the CD4 receptor as well as to one of the recently characterized chemokine coreceptors on target cells[9]. After the gp120–CD4–coreceptor binding, the gp41 subunit undergoes a conformational change that promotes fusion of viral and cellular membranes, resulting in entry of the viral core into the cell, transport to the nucleus and, ultimately, proviral integration and expression[10].

Two regions of HIV gp41 contain consensus motifs predictive of hydrophobic alpha-helices[11,12]. These two 'heptad repeat' sequences have been modeled with synthetic peptides, DP107 and DP178, which were found to associate in a 'coiled-coil' secondary structure[13–17]. Recent crystallographic studies of gp41 fragments show that the two heptad repeat domains form a helical bundle containing three members (a trimer) of each domain[10,18,19]. Studies involving these heptad repeat sequences suggest they have a role in the conformational changes essential for membrane fusion of HIV-1 with host cells[20,21]. This process (Fig. 1) has been compared to the 'spring-loaded' mechanism described for influenza virus in which hemaglutinin changes from a loop structure to an extended coiled-coil, moving a 'fusion peptide' into a favorable position for membrane fusion to occur[22,23]. The synthetic peptide mimics (DP107 and DP178) inhibit infection *in vitro* by disrupting the gp41 conformational changes associated with membrane fusion[17,21,24–26]. A 36-amino-acid peptide, corresponding to DP178 and called T-20, was found to be a particularly potent inhibitor of HIV-1 in T-cell lines (50% inhibitory concentration (IC_{50}) = 1.7 ng/ml)(ref. 24). This agent was proposed as a promising foremost compound for a new class of antiviral drugs[24].

Table 1 Baseline characteristics of subjects

	3 mg (n = 4)	10 mg (n = 5)	30 mg (n = 4)	100 mg (n = 4)	Total (n = 17)
Median age, years (range)	37 (25–47)	37 (30–40)	36 (25–48)	33 (30–36)	36 (25–48)
Gender, male:female	4:0	4:1	4:0	4:0	16:1
Race, Black:White	1:3	2:3	3:1	2:2	8:9
Previously treated: Treatment naive	3:1	5:0	1:3	1:3	10:7
Median days since previous therapy	15 (14–40)	36 (15–210)	30	150	33 (14–210)
Median plasma HIV RNA by bDNA, $\log_{10}$ (range)	4.86 (4.41–5.19)	5.45 (4.23–5.78)	4.76 (4.25–5.71)	3.83 (3.63–4.82)	4.77 (3.63–5.79)
Median absolute $CD4^+$ cells/μl blood (range)	200 (112–350)	279 (105–444)	440 (23–774)	257 (103–658)	212 (23–774)

Preliminary safety and pharmacokinetic studies in animals have indicated that intermittent intravenous T-20 dosing could maintain plasma levels substantially higher than the viral inhibitory concentration (T. Venetta, unpublished data). Here we report the results of the first clinical trial, to our knowledge, examining the safety, pharmacokinetics and anti-retroviral activity of T-20 administered intravenously to HIV-infected humans.

Baseline characteristics

We assessed the baseline characteristics of all 17 subjects enrolled in our study (Table 1). Seven patients had not previously received anti-retroviral therapy and ten had received anti-retroviral therapy before the study. One subject (in the 10-mg dose group) dropped out of the study on day 1 (for personal reasons); thus, another subject was added to the 10-mg dose group before subjects were enrolled in the higher dose groups. Four subjects in each of the four dose groups (total, sixteen subjects) completed the protocol. The median time off of therapy for the ten previously treated patients was 33 days (range, 14–210 days). The median baseline plasma HIV RNA by bDNA assay and CD4 cell counts of the overall study population were 58,884 (4.77 $\log_{10}$) copies per ml and 212 cells per ml, respectively.

Safety and tolerability

Four subjects in each treatment group completed the protocol period as planned except for one subject (10-mg dose group) who left the hospital for one day (day 11) and missed two doses of T-20. No patients were withdrawn from study because of adverse events or toxicity. Four subjects had temperature elevations during the study. The only episodes of fever with temperatures greater than 38.6 °C could have been caused by other clinical events (community-acquired pharyngitis in one subject and phlebitis at the intravenous catheter site in another). One subject in the 3-mg dose group, one in the 30-mg dose group and two in the 100-mg dose group had isolated episodes of mild-to-moderate headaches; an association with T-20 administration was possible. Transient symptoms noted for which a relationship to T-20 was unclear included mild or moderate pain in the extremities (number of subjects with this symptom (n) = 3), lymph node tenderness or swelling (n = 2), subtle skin discoloration (n = 1), dizziness (n = 1), and chest pain (n = 1). There were no clinically relevant alterations in chemistry or hematologic studies during this trial.

Pharmacokinetic measurements

We determined the median pharmacokinetic parameters in each of the dose groups (Table 2). The median $t_{1/2}$ of T-20 was 1.83

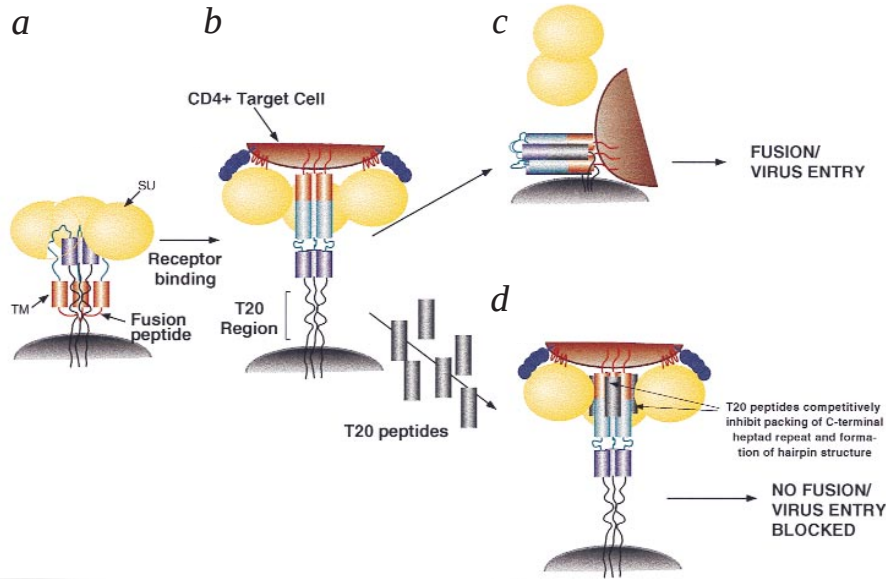

Fig. 1 Proposed mechanism of T-20 action. ***a***, The HIV-1 envelope glycoproteins consist of surface (gp120; SU) and transmembrane (gp41; TM) components. The fusion peptide is in an unexposed position when TM is in the native, non-fusogenic conformation. ***b***, After gp120 binds to a $CD4^+$ target cell, TM changes conformation, 'unfolding' by a hinge mechanism, and the fusion peptide extends away from the viral surface, inserting into the target cell membrane and forming a 'pre-hairpin intermediate' (ref. 49). ***c***, TM resolves into a fusion-active hairpin structure, pulling the cell and viral membranes into close proximity, and allowing fusion and viral entry to occur. ***d***, When T-20 is administered, the drug binds to the highly conserved hydrophobic groove of the heptad repeat portions of TM that normally mediate the conformational change from pre-hairpin intermediate to fusion-active hairpin. This prevents membrane apposition, fusion, and virus entry.

Table 2 Pharmacokinetic measurements: Median (ranges) values after the first (single dose) and seventh (steady-state) intravenous doses of T-20

		3 mg	10 mg	30 mg	100 mg
Maximum observed concentration (μg/ml)	single-dose*	0.37 (0.31–0.84)	1.61 (1.14–3.10)	5.37 (4.04–7.51)	18.30 (17.39–25.93)
	steady-state*	0.39 (0.31–0.73)	1.53 (1.02–2.77)	5.55 (4.98–10.19)	20.67 (19.12–24.62)
Maximum theoretical concentration (μg/ml)	single-dose*	0.45 (0.37–0.79)	1.97 (1.24–3.38)	6.25 (4.88–8.04)	21.23 (19.82–31.72)
	steady-state*	0.43 (0.37–0.61)	1.81 (1.16–3.09)	6.82 (5.60–10.82)	24.10 (23.34–28.85)
Area under curve (μg/hr per ml)	single-dose*	1.19 (0.79–8.73)	4.41 (3.37–9.29)	15.92 (10.83–31.51)	51.70 (48.55–77.05)
	steady-state*	1.11 (0.97–4.62)	4.89 (2.54–10.39)	16.39 (11.87–34.84)	64.43 (48.28–103.12)
Trough concentration (μg/ml)	single-dose*	0.02 (0.01–0.13)	0.01 (0.004–0.03)	0.04 (0.01–0.07)	0.05 (0.02–0.07)
	steady-state*	0.02 (0.002–0.14)	0.05 (0.02–0.37)	0.32 (0.16–0.70)	1.02 (0.92–2.86)
Half-life of T-20 (hr)	single-dose	1.84 (1.50–7.68)	1.69 (1.54–2.29)	1.77 (1.54–2.72)	1.68 (1.54–1.87)
	steady-state	1.81 (1.75–5.28)	1.91 (1.51–2.33)	1.75 (1.22–2.23)	1.85 (1.43–2.48)
Volume of distribution (liter)	single-dose	6.71 (3.82–8.20)	5.08 (2.97–8.09)	4.81 (3.73–6.14)	4.71 (3.15–5.05)
	steady-state	7.01 (4.96–8.08)	5.66 (3.24–8.60)	4.40 (2.77–5.00)	4.15 (3.46–4.29)

*, $P < 0.01$; Kruskal-Wallis Test.

hours, which was relatively stable over time and across all dose groups. The median trough concentration of T-20 at steady-state for patients in the 100-mg dose group was 1.02 μg/ml, which is substantially higher than the *in vitro* inhibitory concentration of the drug (IC_{50} = 1.7 ng/ml)(ref. 24).

Measurements of anti-retroviral activity

There was a significant decline in the level of plasma HIV RNA from day 1 to day 15 of the study when all 16 subjects were considered together (the median change for all dose groups combined was –0.39 $\log_{10}$; $P < 0.05$). We determined the median change in plasma HIV RNA level from baseline for the duration of the study for each treatment group (Fig. 2). There was a significant difference between dose groups with respect to HIV RNA levels over time ($P < 0.001$), indicating a dose–response relationship between the amount of drug administered and the antiviral effect. As predicted from pharmacokinetic studies in animals, the 3-mg and 10-mg dose groups had minimal, if any, changes in HIV RNA levels (–0.08 $\log_{10}$ and –0.05 $\log_{10}$, respectively). The 30-mg dose group had a median decline of –0.62 $\log_{10}$ over the 14 days of intermittent therapy, and in the 100-mg dose group, all four patients had a decline in HIV RNA levels below the detection limit of the bDNA assay (500 copies per ml). These samples were re-tested using an ultrasensitive PCR-based HIV RNA assay (Fig. 3). The median change in plasma viral load in these patients was –1.96 $\log_{10}$ by day 15 of the study, at which time the absolute plasma viral RNA levels were 320, 647, 1197 and 1721 copies per ml for patients A–D, respectively.

We summarized the changes in plasma HIV RNA and CD4+ lymphocyte counts for all 16 patients who completed the study assessments (Table 3). There was a statistically significant decline in plasma viral RNA between day 1 and day 15 for the 100-mg dose group, whether the bDNA or the ultrasensitive PCR assay was used ($P < 0.05$ for both assays). Plasma viral load in this dose group rebounded to near-baseline levels (median 3.73 $\log_{10}$ HIV RNA copies per ml, by bDNA assay) a week after T-20 treatment was stopped (day 21). There was a relatively wide variation in CD4 lymphocyte counts in all dose groups between baseline and day 15, ranging from a decrease of 104 cells to an increase of 177 cells. No statistically significant changes in absolute CD4 counts were observed over the 2-week period of study.

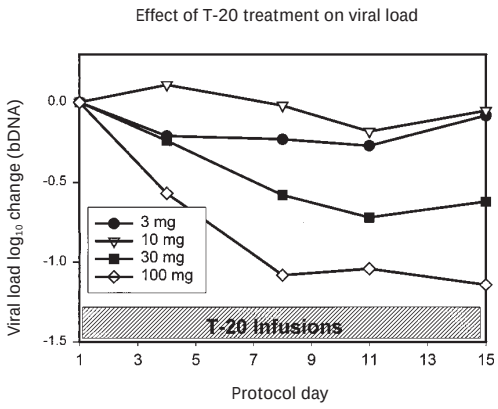

Fig. 2 Median plasma viral load changes from baseline for subjects in all four dose groups: Filled circle, 3 mg; open triangle, 10 mg; filled square, 30 mg; open diamond, 100 mg. Shaded bar, T-20 treatment period. There was a significant difference from baseline on day 15 for all groups ($P < 0.05$), as well as for the 100-mg dose group considered alone ($P < 0.05$). Viral loads measured by bDNA assay[45,46].

Viral dynamics

The substantial declines in plasma virus in the 100-mg dose group warranted a kinetic analysis of virus decline, and by inference, an estimation of the relative antiviral efficacy of the T-20 agent compared with other anti-retrovirals. The kinetics of the initial phase of virus elimination was evaluated during the first week of treatment as described[27–29]. There was remarkable uniformity (0.48–0.56) in the viral decay slopes of the four patients treated with the 100 mg dose. These rates correspond to a composite half-life of 1.4 +/– 0.1 days for plasma virus and virus-producing cells. The magnitude of virus decline during the first phase of elimination was also quite uniform, amounting to a median of –1.52 $\log_{10}$ on Day 8. By day 15, the median reduction in virus load was –1.96 $\log_{10}$ (Fig. 3).

Discussion

This study describes the safety, pharmacokinetics, and anti-retroviral activity of T-20, a new synthetic peptide that blocks HIV-1 gp41-mediated membrane fusion. This was a Phase I/IIB, open-label trial involving 16 subjects treated for a 2-week period. There were no significant drug-related side-effects, and

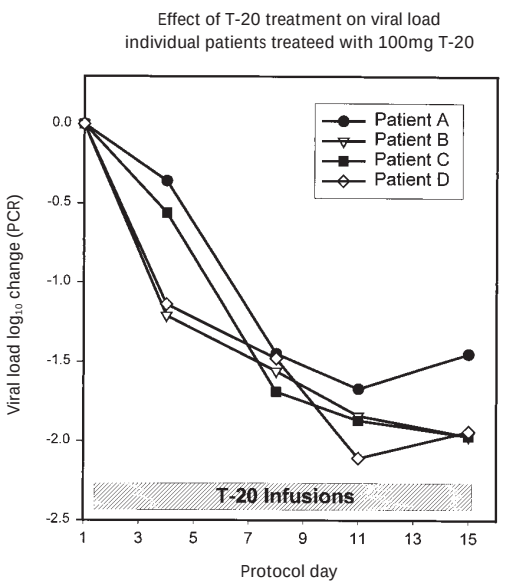

Fig. 3 Plasma viral load changes from baseline for the four subjects in the 100-mg dose group ($P < 0.05$ for difference from baseline on day 15). Filled circle, patient A; open triangle, patient B; filled square, patient C; open diamond, patient D. Shaded bar, T-20 treatment period. Viral loads measured by ultrasensitive PCR assay[47].

the anti-retroviral activity of the agent was unequivocal and of substantial magnitude (–1.96 $\log_{10}$ reduction in plasma viral load at the highest dose level). This study thus represents the first clinical demonstration, to our knowledge, that selective inhibition of HIV-1 fusion and entry in humans can lead to biologically significant reductions in plasma virus load. The findings provide proof-of-concept for new therapeutics targeting this essential step in the life-cycle of HIV and other fusogenic, enveloped viruses.

The magnitude and kinetics of virus decline indicate that the efficiency of suppression of *de novo* virus infection by T-20 is comparable to that achieved with anti-retroviral treatments available now[2–4,27–31]. Here, administration of T-20 led to a fall in plasma HIV RNA of about 2 $\log_{10}$ (99%) with a half-life of 1.4 ± 0.1 days. The slope of virus decline is a measure of the anti-retroviral potency of a drug[32,33]; a more potent drug should lead to a faster decline. Monotherapy with protease inhibitors has resulted in viral decay half-lives of 1.8 ± 0.9 days[27], 2.1 ± 0.4 days[28], and 1.6 ± 0.6 days[30] for productively infected cells. Triple-drug therapy using two reverse transcriptase inhibitors and one protease inhibitor led to a half-life of 1.1 ± 0.4 days[34]. Even studies involving drug-naive patients treated with four-drug regimens of highly active anti-retroviral therapy (saquinavir, ritonavir, zidovudine and lamivudine; amprenavir, abacavir, zidovudine and lamivudine) showed slopes of plasma virus decline of 0.43/day and 0.44/day, respectively, corresponding to composite plasma virus and productive cell half-lives of 1.72–1.80 days (D. Ho, personal communication and abstract S49, 5th Conference on Retroviruses and Opportunistic Infections, Chicago, Illinois, 1998).

Our findings indicate that over a 2-week treatment period, the efficacy of T-20 in blocking *de novo* virus infection is comparable to that of protease and RT inhibitors. After 14 days of therapy, virus was still detectable in the plasma of each patient, even those receiving the highest dose of T-20. This observation is analogous to the results with the highly active combination regimens now available, for which the time necessary to achieve plasma HIV RNA levels less than 25–50 copies per ml is typically longer than 12 weeks[31,34]. The similarity, in kinetics and the magnitude of virus decline, between T-20 and agents now available demonstrates that they are equally accessible to the principal sites of virus replication responsible for sustaining steady state plasma virus loads, which are prognostically very important.

Other strategies have been pursued in an attempt to inhibit viral entry. Sulfated polyanions, such as dextran sulfate or heparin, inhibit HIV infection *in vitro* apparently because of nonspecific interference with HIV glycoprotein binding to lymphocytes[35,36]. However, studies of dextran sulfate in humans demonstrated substantial toxicity and resulted in paradoxical increases in virus load[37]. Soluble CD4 and CD4-IgG also effectively inhibit HIV replication *in vitro*[38], but clinical trials of these agents did not demonstrate consistent anti-retroviral activity[39–41]. These shortcomings may have been due to differences between laboratory-adapted and primary isolates of HIV-1 or the presence of factors in plasma that inhibit the activity of these agents *in vivo*, or they may relate to the binding of gp120 to secondary cellular HIV-1 receptors in addition to the CD4 receptor. Strategies aimed at blocking the interaction of HIV-1 gp120 with the CXCR4 chemokine receptor (which preferentially binds to syncytium-inducing viral isolates associated with advanced or rapidly progressive HIV infection) were promising in preliminary *in vitro* studies[42,43]. Our results with T-20 provide additional support for exploring agents that target membrane fusion and virus entry; future investigations will be further aided by recent detailed characterization of the viral envelope glycoprotein structure and binding characteristics[9].

The development of a suitable outpatient drug delivery system for T-20 could result in substantial clinical benefit as a result of inhibiting this additional step in the viral life cycle. Despite the promising anti-retroviral activity in this trial, the complexities involved in developing a suitable oral formulation for peptide-based agents may initially limit the widespread clinical application of T-20. Studies with insulin, another par-

Table 3 Effects of T-20 treatment

	Viral load		CD4 counts	
Dose group	Day 1 viral load (median $\log_{10}$; range)	Median change on day 15 ($\log_{10}$; range)	Entry[a] CD4 count (median cells/μl; range)	Median change on day 15 (range)
3 mg (bDNA)	4.86 (4.41, 5.19)	-0.08 (–0.40, +0.11)	222 (144, 392)	-27 (–93, +4)
10 mg (bDNA)	5.45 (4.23, 5.78)	–0.05 (–0.21, +0.31)	324 (125, 463)	+24 (–104, +177)
30 mg (bDNA)	4.76 (4.25, 5.71)	–0.62 (–0.77, +0.53)	400 (115, 814)	-11 (–40, +46)
100 mg (bDNA)	3.83 (3.63, 4.82)	-1.14[b,c] (–2.13, -0.93)	267 (119, 589)	+80 (–13, +109)
100 mg (PCR)	4.74 (4.45, 5.05)	–1.96[c] (–1.97, –1.45)		

[a]Mean of screening and day 1 values [b]All values less than 500 copies/ml on bDNA assay [c]Difference from baseline, $P < 0.05$

enterally administered protein, have demonstrated controlled plasma pharmacokinetics and improved clinical outcomes when the drug is delivered by continuous subcutaneous administration[44]. The pharmacokinetic profile of T-20 (plasma $t_{1/2}$, 1.7–2.0 hours) indicates that either intermittent or continuous parenteral administration may be possible. Larger, multicenter, randomized trials are in development. These studies will evaluate the safety, pharmacokinetics and antiviral activity of T-20 when it is given by continuous subcutaneous infusion as a component of a 'rescue regimen' for patients who have had virologic failure while receiving anti-retroviral therapies now available. HIV isolates with reduced susceptibility to T-20, derived from serial *in vitro* viral passage in the presence of the compound, have been characterized[26]. Further clinical studies are underway to assess the potential for selection of variants resistant to T-20 *in vivo* as well as the possibility that anti-T20 antibody responses could alter the efficacy of the compound. Nonetheless, blocking of membrane fusion and viral entry by T-20 establishes a new class of antiviral therapy that exhibits potent activity *in vivo* and is unlikely to be cross-resistant with currently available agents.

Methods

Study patients. Adult HIV-infected subjects were screened at the University of Alabama at Birmingham (UAB) 1917 Clinic, and were eligible for this study if they had at least two viral load determinations of 10,000 or more copies of HIV-1 RNA per ml of plasma as determined with the branched-chain DNA assay (Chiron, Emeryville, California)[45,46]; a CD4 count greater than or equal to 100 cells per mm^3; and either never received anti-retroviral therapy or had discontinued all anti-retroviral therapy at least 14 days before starting the medication for this study. Patients were excluded if they had a poor clinical status (Karnofsky score less than 80%), clinically unacceptable screening hematologic or blood chemistry results, or signs of an active opportunistic infection or invasive neoplasm . All subjects provided informed consent for participation in the study which was approved by the UAB institutional review board.

Study design and patient evaluation. This study was a single-site, open-label, dose-escalation trial of single-agent therapy with intravenous T-20 administered in the General Clinical Research Center (GCRC) in-patient unit at UAB. We planned to study four patients at each dose level (3 mg, 10 mg, 30 mg and 100 mg, all twice daily) and add as many as four more patients at any dose level if significant toxicity or intolerance were encountered. Two baseline viral load determinations and two CD4 counts were obtained during the 30-day screening period. All patients who were receiving anti-retroviral therapy before the trial underwent a 'washout' phase lasting at least 14 days. Each patient received a single intravenous infusion of T-20, at his or her respective dose level, 3 days before beginning the 2-week daily dosing schedule (day –3). This initial test dose was used for preliminary safety and pharmacokinetics assessments. On day 1, patients began intermittent T-20 infusions, for 20 minutes every 12 hours for 14 consecutive days (days 1–14). Vital signs, physical examinations, blood chemistries, and complete blood counts were monitored during the study. Blood for viral load determinations was obtained on days –14, –3, 1 (before T-20 dosing), 4, 8, 11, 15 and 21; $CD4^+$ lymphocyte counts were determined on days –14, 1, 4, 8 and 15. Patients were discharged from the hospital on day 15 and returned for an outpatient follow-up visit on day 21.

Virologic measurements

Anti-retroviral activity was first assessed by measuring plasma viral load using the branched-chain DNA method (bDNA; Chiron, Emeryville, California)[45,46], which has a detection limit of 500 copies of HIV-1 RNA per ml. Because viral RNA levels fell below detectability by the bDNA assay in all four patients who received the highest dose of T-20 (100 mg), plasma samples from these patients were re-tested using a quantitative ultrasensitive RT–PCR HIV RNA assay[47] (Roche, Somerville, New Jersey) with a detection limit of 40 copies per ml.

Pharmacokinetic studies

Frequent blood sampling was obtained twice, on day –3 and on day 3, for single-dose and steady-state pharmacokinetic studies, respectively. Blood samples on these days were drawn immediately before and 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after the completion of the 20-minute drug infusion. T-20 levels were determined using a sandwich capture ELISA assay (T.M.V., manuscript in preparation).

Statistical analysis

We used the Wilcoxon rank sum test to compare dose levels with respect to CD4 count and viral load at baseline, and the paired t-test to compare values from day 1 with those from day 15 for CD4 count and viral load within each dose level. We evaluated the effect of T-20 dose groups and time on study on the CD4 count and viral load using 2-way analyses of variance. Pharmacokinetic data were fit to a one-compartment model utilizing SAS non-linear procedures[48]. Area under the curve, half-life, and the volume of distribution were calculated using the estimated coefficients derived from the non-linear regression procedure. All pharmacokinetic measurements, viral load results and CD4 counts are expressed as medians with the corresponding ranges in parentheses; changes in the last two parameters are expressed as median change from baseline.

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