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CGP 53437, an Orally Bioavailable Inhibitor of Human Immunodeficiency Virus Type 1 Protease with Potent Antiviral Activity†

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CGP 53437 is a peptidomimetic inhibitor of human immunodeficiency virus type 1 (HIV-1) protease containing a hydroxyethylene isostere. The compound inhibited recombinant HIV-1 protease with a K_i of 0.2 nM. The inhibition constant versus human cathepsin D and human cathepsin E was 4 nM. Human pepsin and gastricsin were inhibited with K_is of 8 and 500 nM, respectively, and human renin was inhibited with a K_i of 190 μM. The replication of HIV-1/LAV, HIV-1/Z-84, and HIV-1/pLAI was inhibited with a 90% effective dose of 0.1 μM in acutely infected MT-2 cells. The 50% cytotoxic dose was 100 μM. Similar antiviral activity was observed when the compound was added up to 10 h after infection. At the effective concentration, processing of Gag precursor protein p55 was greatly reduced, confirming an action on the late stage of the virus life cycle, as expected. The efficacy of the inhibitor was also demonstrated by using primary human peripheral blood lymphocytes infected with the HIV-1/LAV strain, low-passage clinical isolates obtained from HIV-1-seropositive individuals (including a zidovudine-resistant strain), and HIV-2/ROD. In these cells, CGP 53437 delayed the onset of HIV replication in a dose-dependent fashion (substantial effects with concentrations of ≥0.1 μM) as long as the inhibitor was maintained in the culture. CGP 53437 was orally bioavailable in mice. Concentrations in plasma 10-fold in excess of the in vitro antiviral 90% effective dose could be sustained for several hours after oral application of 120 mg/kg. Therefore, CGP 53437 has the potential to be a therapeutically useful anti-HIV agent for the treatment of AIDS.

Human immunodeficiency virus (HIV) replication is dependent on the cleavage of two precursor proteins, p180gag-pol and p55gag, by the virally encoded protease. This viral protease has been identified as an attractive target for antiviral interference. Several types of HIV type 1 (HIV-1) protease inhibitors have been described (for a review, see reference 17). These can be grouped into three classes: (i) nonpeptidic compounds (such as cerulenin and haloperidol) (3, 6), (ii) peptidomimetics containing a nonhydrolyzable substitution for the P1-P1′ scissile bonds present in the natural substrates (7, 23), and (iii) C_2 symmetric structures which were designed to fit the symmetric active center of the enzyme (9). Compounds of all of these classes exhibited potent anti-HIV-1 activity in in vitro cell assays with acutely and chronically infected cell lines or primary cells (5, 18, 19). However, the high lipophilicity or poor aqueous solubility of some of these compounds is a major drawback that presented difficulties for the in vivo characterization and precluded their further development (13). In this report, we describe the biological profile of the HIV-1 protease inhibitor CGP 53437, which belongs to the peptidomimetic class. This compound not only displays broad and potent antiviral activity against HIV-1 but is also orally bioavailable and therefore suitable for clinical studies.

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MATERIALS AND METHODS

Enzymatic assays. Recombinant HIV-1 and HIV-2 proteases were expressed and purified as previously described (25). The inhibition constant of CGP 53437 versus HIV-1 protease was determined spectrophotometrically at 300 nm in 0.1 M sodium acetate buffer, pH 4.7, containing 4 mM EDTA and NaCl at a final ionic strength of 0.3 M (22). The chromophoric peptide substrate used had the amino acid sequence KARINle*NphEANleNH$_2$. The K_i versus HIV-2 protease was determined with the same buffer system by using the chromophoric peptide substrate KARVNle*Nph EANleNH$_2$. The reactions were started by addition of 5 to 15 nM HIV-1 or HIV-2 protease and incubated at 37°C. The source of human pepsin, gastricsin, and cathepsins D and E was previously described (8, 12). The K_i values for CGP 53437 against human pepsin, gastricsin, and cathepsins D and E were determined in 0.1 M sodium formate buffer, pH 3.1, with the chromophoric substrate KPIEF*NphRL (11). Isolation of human renin and the enzymatic assay conditions used have been described previously (29).

Primary cells and cell lines. Peripheral blood lymphocytes (PBLs) were obtained from the blood of HIV-seronegative volunteers by leukapheresis as previously described (16). The MT-2 cell line was a gift from M. Meltzer, Walter Reed Army Institute of Research, Washington, D.C. The A3.01 cell line was a gift from M. Martin, National Institutes of Health, Bethesda, Md. Both cell lines were maintained in culture in RPMI 1640 (GIBCO, Paisley, Scotland) supplemented with 10% fetal calf serum (SEROMED, Berlin, Germany), 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 2 mM L-glutamine

(AMIMED, Muttenz, Switzerland). The cells were mycoplasma free as assessed by the GenProbe Hybridization Assay (Gen-Probe, San Diego, Calif.).

Compounds. The design and synthesis of CGP 53437 (Boc-PheΨ[CH(OH)CH_2]Phe-Val-Phe-morpholine) is described elsewhere (1). 3'-Azido-3'-deoxythymidine (AZT; Sigma Chemical Co., St. Louis, Mo.) was dissolved to the desired concentration in complete medium without fetal calf serum. CGP 53437 was dissolved in 100% dimethyl sulfoxide at 2 mM and diluted to the desired final concentration in complete medium without fetal calf serum. The concentration of dimethyl sulfoxide in the cellular assays was less than 0.5%.

Virus strains. HIV-1/LAV (2) was a gift from M. Martin and was expanded in A3.01 cells. HIV-1/Z-84 (31) was a gift from M. Martin and was expanded in phytohemagglutinin (PHA)-blasted PBLs. Infectious HIV-1 proviral clone pLAI (27) was a gift from K. Peden. This plasmid was transfected into HeLa/$CD4^+$ cells and propagated in PHA-blasted PBLs. HIV-2/ROD (10) was originally obtained from the Pasteur Institute, Paris, France, and expanded at the Central Laboratory of the Netherlands Red Cross, Amsterdam. All viral stocks were kept in aliquots at −80°C, and a fresh aliquot was thawed for each experiment. The titers of the stocks were determined as 50% tissue culture-infectious doses ($TCID_{50}$s) on MT-2 cells by measuring the supernatant reverse transcriptase (RT) activity after 5 days of culture. Calculation of the titer was done with the Spearman-Karber formula (20).

Virus isolates. Low-passage clinical isolates H-638, H-672, and H-688 were obtained from HIV-1-seropositive individuals and provided by C. Moroni (Institut für Medizinische Mikrobiologie, Basel, Switzerland). AZT-resistant HIV-1 strain AMS316-23 was isolated and expanded at the Central Laboratory of the Netherlands Red Cross.

Acute infection of MT-2 cells with HIV-1/LAV, HIV-1/Z-84, and HIV-1/pLAI. Tests were performed with MT-2 cells seeded in 96-well round-bottom plates (Nunc, Kamstrup, Denmark) at 4×10^4 per well in 50 μl of complete medium. The compounds were added in a volume of 50 μl, and the virus (360 $TCID_{50}$s per well) was added in a volume of 100 μl. The assays were performed in triplicate. After 4 to 7 days (depending on the virus used) of incubation at 37°C in a fully humidified atmosphere containing 5% CO_2, 10-μl samples of the culture supernatants were collected and stored frozen at −20°C until assayed for RT activity.

Acute infection of PBLs with HIV-1/LAV or low-passage clinical isolates of HIV-1. PHA-stimulated PBLs were infected with LAV or low-passage clinical isolates as previously described (15). Briefly, PBLs from healthy volunteers were cultured for 2 days in the presence of PHA (0.25 μg/ml) prior to infection. Cells were infected with virus-containing supernatants that were normalized on the basis of RT activity (approximately 1,000 cpm/μl). The cells were exposed to HIV-1 for 6 h, and then free virus was removed and the cells were further cultured at 0.6×10^6/ml in the presence of interleukin 2 for 10 days. Medium was changed on days 3 and 6. During this period, no fresh PBLs were added. The compound under test was added to the culture immediately after infection and again with the first medium change, on day 3 postinfection.

Acute infection of PBLs with AZT-resistant HIV-1 isolate AMS316-23 and HIV-2/ROD. Assays were performed at the Central Laboratory of the Netherlands Red Cross. PHA-stimulated PBLs (2×10^7) were inoculated with 2×10^3 $TCID_{50}$s of HIV-1 isolate AMS316-23 or HIV-2/ROD in a total volume of 2 ml. After 6 h, cells were carefully washed in a total volume of 30 ml to remove unadsorbed virus. Cells were then resuspended in 20 ml of culture medium (Iscove's modified Dulbecco medium supplemented with 10% fetal calf serum, 5 μg of Polybrene per ml, 5 μg of PHA per ml, 100 U of penicillin per ml, 100 μg of streptomycin per ml), and from these suspensions, 2-ml aliquots, containing 2×10^6 cells, were transferred to six new tubes. Dilutions of CGP 53437 in culture medium were added, and cells were transferred to a 24-well plate (Nunc) at 10^6/ml per well. Medium was changed on days 3, 7, 10, and 14. On these days, a new dose of inhibitor was added to the cultures. At day 14, 10 μl of culture supernatant was harvested for determination of RT activity and 125 μl was harvested for $TCID_{50}$ determination on PHA-stimulated PBLs.

Immunoblotting. MT-2 cells were harvested and lysed in Laemmli sample buffer (14). Proteins were separated on a sodium dodecyl sulfate-polyacrylamide gel, transferred onto an Immobilon membrane (Millipore, Bedford, Mass.), and probed with a mouse monoclonal antibody (generously provided by H. K. Hochkeppel) directed against HIV-1 p24; a horseradish peroxidase-coupled goat anti-mouse antibody (Bio-Rad, Richmond, Calif.) was used as the second antibody. The blot was then developed with 4-chloro-1-naphthol as the substrate.

Virus detection. Virus production was determined as virion-associated RT activity in the supernatants of infected cells. RT activity was measured as described previously (28), with the following modifications. The reaction mixture was adjusted to 0.1% Nonidet P-40, 0.8 mM EDTA, and 10 μg of poly(A) per ml, with 0.16 μg of oligo(T) per ml as the template primer. The assay was performed in a 96-well plate format.

$TCID_{50}$ determination. The supernatants from HIV-1/LAV-infected MT-2 cells (some were treated with CGP 53437 at various concentrations, and some were untreated) were collected on day 4 and stored at −20°C until assayed. MT-2 cells (4×10^4) were seeded in a round-bottom 96-well plate at 100 μl per well. The supernatants were added in a volume of 100 μl in serial 1:10 dilutions, with six replicate wells per dilution. After 5 days of incubation, supernatants were tested for RT activity and $TCID_{50}$s were calculated by the Spearman-Karber formula. The supernatants from HIV-1 isolate AMS316-23- or HIV-2/ROD-infected PBLs (untreated or treated with various concentrations of CGP 53437) were tested in triplicate fivefold dilutions on PHA-stimulated PBLs. For each dilution, 25-μl volumes were mixed with 100 μl of culture medium. From each dilution step in all series, 25 μl was mixed with 10^5 PHA-stimulated PBLs in 100 μl of culture medium, seeded in a 96-well plate, and cultured at 37°C in a humidified atmosphere with 5% CO_2. Twice a week, cultures were refed with fresh medium and virus production was evaluated by the occurrence of a cytopathic effect and the presence of RT activity.

Viability test. The 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) test was used to assess the cytotoxicity of the compound (24). XTT was obtained from Polysciences (Warrington, Pa.). Viable cells metabolize this reagent to a soluble product with an optical density readable in the 450-nm band. At the end of the standard 4-day MT-2 cell assay (see above), fully viable cells give optical density readings between 1.5 and 2.

Animal studies. CGP 53437 was dissolved in dimethyl sulfoxide at 40 mg/ml for intravenous (i.v.) administration and at 240 mg/ml for per oral (p.o.) administration. These

solutions were diluted 1:20 with 20% (wt/vol) hydroxypropyl-β-cyclodextrin (Wacker Chemie, Munich, Germany) in water to give a final solution of 2 mg/ml for i.v. administration and a final homogeneous suspension of 12 mg/ml for p.o. administration. Twenty-eight-day-old female BALB/c mice (BALB/c AnCbif Tif [specific pathogen free]) were used for pharmacokinetic studies. They had free access to food and water throughout the experiment. The compound was either injected into the tail vein or administered by gavage. In both cases, 0.2 ml per mouse was administered, giving average doses of 20 mg/kg i.v. and 120 mg/kg p.o. Four or five mice were used per time point, and at the allotted times retroorbital blood was collected into heparinized tubes from animals under enfluran anesthesia. Mice were not allowed to recover from anesthesia.

Analytical procedures. Mouse blood samples (0.5 ml) were spiked with an internal standard. The blood samples were centrifuged (10,000 × *g*, 5 min), and the plasma was removed and mixed with an equal volume of acetonitrile (Merck). The protein precipitate was removed by centrifugation (10,000 × *g*, 5 min), and the supernatant was dried under a vacuum. The residue was resuspended in 0.1 ml of a 0.05 M phthalate buffer (pH 3) and 20 μl of 3 M NaCl. The mixture was extracted twice, with 1 and 0.2 ml of diisopropyl ether (Merck). The diisopropyl ether fractions were pooled and evaporated under a vacuum. The residue was dissolved in 50% acetonitrile in water (Baker) before analysis by reversed-phase high-pressure liquid chromatography, which was carried out on a Nucleosil C18 analytical column (125 by 4.6 mm) equilibrated with a mobile phase of 50% acetonitrile, 50% water, and 0.1% trifluoroacetic acid. The flow rate was 1 ml/min. Under these conditions, CGP 53437 had a retention time of approximately 15 min and the limit of detection was 0.2 μM. The compound added to mouse blood and processed similarly was used to construct a standard curve for quantitation of the in vivo concentrations.

RESULTS

In vitro enzyme inhibition. The potency of the inhibitor against the viral enzyme was demonstrated in peptide-based enzyme assays using purified recombinant HIV-1 or HIV-2 protease and the chromophoric peptide substrate. The K_i for CGP 53437 against HIV-1 protease was 0.2 nM, and that against HIV-2 protease was 0.56 nM. The selectivity profile of CGP 53437 was determined against other human aspartic proteases. The compound inhibited cathepsins D and E with a K_i of 4 nM and pepsin with a K_i of 8 nM. Human gastricsin was only weakly inhibited by CGP 53437, with a K_i of 0.5 μM. Human renin was only slightly inhibited by the compound (K_i, 190 μM).

Effect on acute infection of MT-2 cells with HIV-1/LAV, HIV-1/Z-84, and HIV-1/pLAI. Figure 1 shows the effect of CGP 53437 on virus production in MT-2 cells. CGP 53437 exhibited antiviral effects against HIV-1/LAV at concentrations above 0.003 μM. At a concentration of 0.1 μM, approximately 90% inhibition of supernatant RT activity was measured. This correlated with a 1,000-fold reduction of infectious titers. At concentrations of 1 and 10 μM, when the RT activity was reduced by more than 90% the infectivity of the corresponding 4-day culture supernatants was completely abolished.

HIV-1/Z-84 and HIV-1 clone pLAI were also able to infect MT-2 cells, showing a peak of RT release later than that seen with HIV-1/LAV. 0.1 μM concentration of the compound was sufficient to reduce supernatant RT activity by more than 90% on day 7 for both viruses. The cytotoxic effect of the compound on noninfected cells was determined by the XTT assay. No cytotoxicity was observed with concentrations up to 10 μM. At 100 μM CGP 53437, 50% of the cells were killed (50% cytotoxic dose).

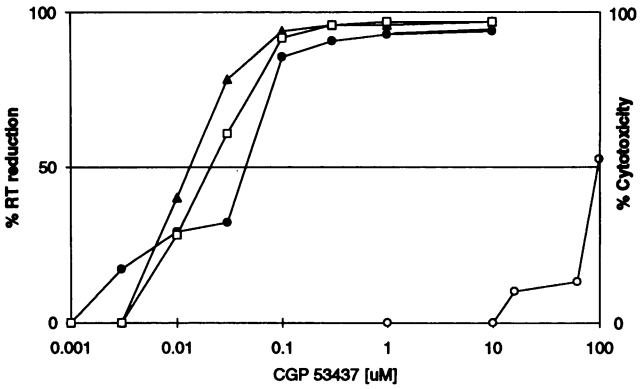

FIG. 1. Dose-response curve for CGP 53437 in acutely infected MT-2 cells. Cells (4×10^4 per well) were seeded in a 96-well plate and infected with 360 $TCID_{50}$s of HIV-1/LAV (●), HIV-1/Z-84 (▲), or HIV-1/LAI (□) per well in the presence of various drug concentrations. Supernatant RT activity was measured in HIV-1/LAV-infected cultures on day 4 and in HIV-1/Z-84- or HIV-1/LAI-infected cultures on day 7 postinfection. The data are presented as percent reduction of RT activity compared with untreated, infected controls. The supernatant RT activity of infected, untreated control cells was approximately 2,000 cpm/μl. Cytotoxicity of the compound was determined with the XTT assay by comparing the optical density at 450 nm of noninfected cells exposed to CGP 53437 with that of noninfected controls (○). Results of triplicate determinations are shown. The standard errors of the means did not exceed 10%.

Effect on replication of HIV-1/LAV and different HIV-1 isolates in PBLs. In the absence of an inhibitor, PBLs infected with HIV-1/LAV or clinical HIV-1 isolates produced peak virus values on day 6 (Fig. 2). At 0.01 μM, CGP 53437 did not have a significant effect on virus replication. At a concentration of 0.1 or 1 μM CGP 53437, virus production was suppressed during the first 6 days and only low levels of virus production were detected at day 10. When cultures were maintained for longer periods (16 days) without further addition of the compound, reemergence of the virus was observed (data not shown).

Effect on replication of AZT-resistant HIV-1 isolate AMS316-23 and HIV-2/ROD in PBLs. AZT-resistant isolate AMS316-23 was susceptible to inhibition by CGP 53437 (Table 1). A 0.1 μM concentration of the compound reduced RT activity by almost 90%, and the $TCID_{50}$ was reduced by more than 2 orders of magnitude. On the basis of the reduction of supernatant RT activity, CGP 53437 exhibited antiviral effects against HIV-2 at concentrations above 0.5 μM. A 50% effective dose of approximately 0.8 μM was determined by graphical interpolation. At 1 μM, the highest dose tested, supernatant RT activity was reduced by 74% but the corresponding $TCID_{50}$ of the culture supernatant was reduced more than 600-fold.

CGP 53437 did not interfere with PHA-interleukin 2-stimulated proliferation of noninfected PBLs (data not shown).

Intracellular processing. We examined the effect of the compound on the processing of viral precursor protein p55. MT-2 cells were seeded at 2×10^5 per well in 1 ml in a 24-well plate, exposed to the drug, and infected with HIV-1/LAV as described in Materials and Methods. After 4

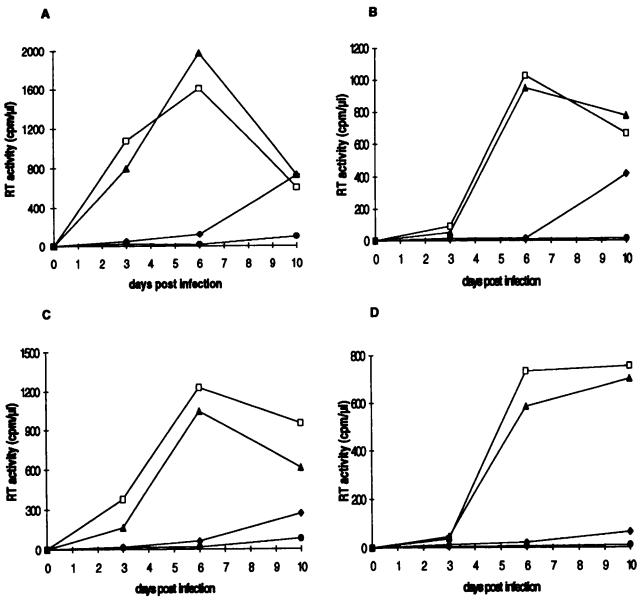

FIG. 2. Effect of CGP 53437 on HIV-1 replication in human PBLs infected with HIV-1/LAV (A) or low-passage clinical isolate H672 (B), H688 (C), or H638 (D). Experiments were performed as described in Materials and Methods. The drug concentrations tested were 1 (●), 0.1 (◆), and 0.01 (▲) and 0 (control [□]) μM. Mean values of quadruplicate determinations are shown. The variability was less than 5%.

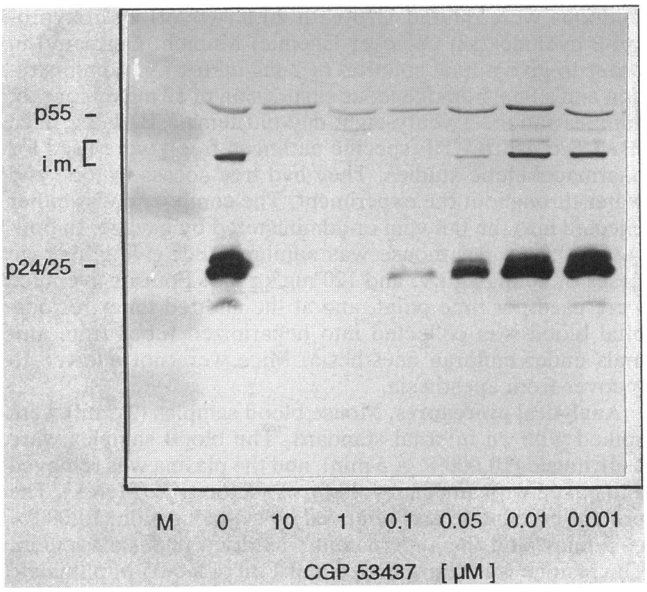

FIG. 3. Immunoblot analysis of lysates from HIV-1/LAV-infected MT-2 cells. Cells were treated with various concentrations of CGP 53437. The blot was developed with a mouse anti-p24 monoclonal antibody. i.m., processing intermediates. Lane M contained molecular size standards.

days, the cells were harvested and lysed. Figure 3 shows an immunoblot analysis of the cell lysate. The protein pattern indicates the presence of p55 at all of the drug concentrations tested. Full inhibition of p55 processing was observed with 1 μM or higher concentrations of CGP 53437. With 0.1 to 0.001 μM CGP 53437, increasing amounts of p24gag protein were detected.

Effect of delayed drug addition. The antiviral potency of CGP 53437 was determined in HIV-1/LAV-infected MT-2 cells when it was added at various time points after infection. When 1 μM CGP 53437 was added up to 10 h after infection, viral replication was reduced by at least 90%. When AZT was tested under the same experimental conditions at a concentration of 15 μM, >90% reduction of supernatant RT activity was observed only if the drug was added no later than 4 h after infection. If AZT was added 10 h after infection, supernatant RT activity was reduced by less than 60%, and if it was added 24 h postinfection, only a marginal antiviral effect was observed (data not shown).

Pharmacokinetics. Figure 4 shows the concentrations of CGP 53437 in plasma after administration of 20 mg/kg i.v., and 120 mg/kg p.o. to mice. Immediately after i.v. administration of 20 mg/kg, the drug concentration was approximately 16 μM. One hour after administration, the drug concentration was below the detection limit (0.2 μM). One hour after p.o. administration of 120 mg/kg, a concentration

TABLE 1. Effect of CGP 53437 on the replication of an AZT-resistant isolate of HIV-1 and HIV-2/ROD in PBLs[a]

CGP 53437 concn (μM)	% Reduction of RT activity		$TCID_{50}$s/ml[b]	
	HIV-2/ROD	AMS316-23	HIV-2/ROD	AMS316-23
0	0[c]	0[d]	$10^{4.9}$	10^{4}
0.01	2	0	$10^{3.0}$	$10^{4.3}$
0.1	0	89	$10^{2.4}$	$10^{1.6}$
0.5	21	92	$10^{2.6}$	$<10^{0.8}$
1	74	89	$10^{2.1}$	$<10^{0.8}$

[a] Average results of six assays are shown.
[b] Infectivity in the supernatant of PHA-stimulated PBLs infected with 10^3 $TCID_{50}$s of AZT-resistant isolate AMS316-23 or HIV-2/ROD.
[c] 1,031 cpm/μl.
[d] 2,203 cpm/μl.

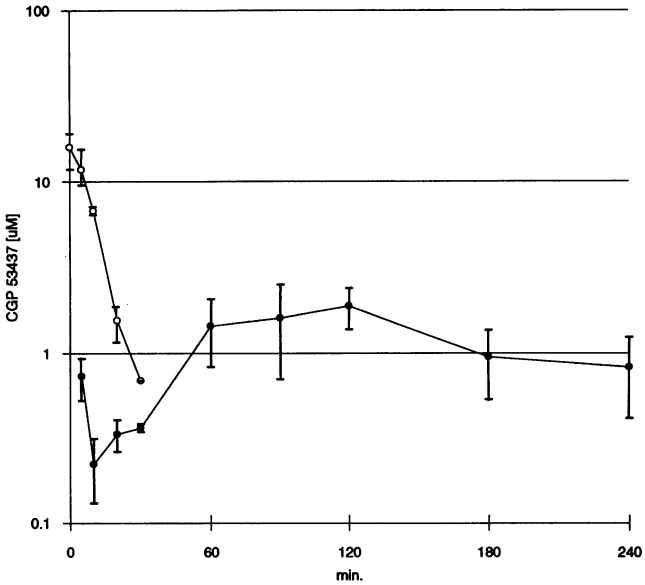

FIG. 4. Concentrations of CGP 53437 in plasma after i.v. administration of 20 mg/kg (○) or p.o. administration of 120 mg/kg (●) to mice. The bars indicate standard errors of the means (n = 4 or 5).

of 1.5 μM was detected. The concentration remained constant during the next hour and then declined slowly. At 4 h after administration, a concentration of 0.8 μM was still detectable, representing approximately eight times the antiviral 90% effective dose determined in MT-2 cells. No signs of toxic effects were observed in these experiments.

DISCUSSION

HIV-1 protease has been recognized as an attractive target for chemotherapeutic intervention in the treatment of AIDS (21). However, the desirable properties of a clinically useful agent—oral administratability, low toxicity, and high efficacy—have not always been met. In this report, we present preclinical data for a compound which appears to fulfill these requirements.

CGP 53437 is highly active against the proteases of HIV-1 and HIV-2. It has no significant effect on the key cardiovascular enzyme renin (30). Low levels of inhibitory activity against lysosomal enzyme cathepsin D and endosomal enzyme cathepsin E were observed. These enzymes are relevant for several immunological functions (4, 26). It has recently been demonstrated that an HIV protease inhibitor with activity against these enzymes did not impair macrophage functions (4). However, before completion of animal toxicology studies, we cannot exclude the possibility that this cross-reactivity will lead to toxicity. Under physiological conditions, human pepsin and gastricsin could be targets for CGP 53437. However, appropriate formulation of the compound might circumvent this inhibition.

To validate the mechanism of action of CGP 53437 at the cellular level, immunoblot experiments demonstrating inhibition of processing of viral precursor proteins were performed. This inhibition of processing correlated well with the antiviral dose response of CGP 53437 in HIV-1/LAV-infected MT-2 cells. It also correlated with the interference of CGP 53437 with virus replication when the drug was added late after infection. Furthermore, the prevention of virus maturation and the subsequent reduction in virus replication were documented in several systems and by independent readout systems, i.e., supernatant RT and p24 quantitation and determination of infectious titers and a cytopathic effect (data not shown). In most studies, the antiviral effect of CGP 53437 is presented as a reduction of supernatant RT activity, and a reduction of 90% or more was considered virologically relevant since these supernatants were not infectious. The antiviral activity of CGP 53437 against HIV-2 was also demonstrated. This activity was less pronounced than that against HIV-1. Such an effect might be explained by the lower drug susceptibility of the enzyme or other fundamental differences between these two viruses. In all cases, strong antiviral effects were observed at concentrations of the compound which did not impair cell viability, cell proliferation, or cellular activity. Interestingly, the concentration of the drug required for viral suppression in cellular systems is generally higher than that required to achieve similar effects at the enzymatic level. This difference between the two systems could be due to factors such as drug stability, uptake, compartmentalization, and metabolism, etc. However, antiviral concentrations eight times the 90% effective dose in MT-2 cells can easily be reached in plasma, as demonstrated by pharmacokinetic studies with mice treated orally with CGP 53437.

Several HIV-1 protease inhibitors with antiviral activity have been described (17). In most instances, data were generated by use of HIV-susceptible human cell lines and expressed as 50% inhibitory endpoints. Such limited information can be misleading because the rate and yield of virus production differ among cell types, as do drug uptake and degradation. In this study, we demonstrated that CGP 53437 has an antiviral effect in suppressing viral replication by 90%, not only in a routinely used cell line such as MT-2 but also in primary human PBLs infected with a broad spectrum of laboratory and low-passage clinical isolates, including an AZT-resistant HIV-1 isolate and HIV-2. Furthermore, preliminary findings suggest that CGP 53437 can inhibit production of infectious virions in chronically infected cell lines and primary macrophages.

Besides the desirable in vitro antiviral profile, CGP 53437 given orally to mice resulted in concentrations in plasma in excess of the antiviral 90% effective dose for an extended period of time. No calculation of bioavailability was undertaken because of the low sensitivity of the detection method for CGP 53437. It is clear, however, that in terms of peak concentrations oral bioavailability is relatively low. The long-lasting concentrations in plasma following p.o. administration may reflect either a slow rate of absorption of the drug or the slow release of the compound from tissues. The results obtained after i.v. administration argue for fast penetration into tissues. Which tissues are involved and the relevance of these findings are under investigation. These in vivo studies were conducted with a formulation which we are currently improving with the aim of making CGP 53437 a clinically useful anti-HIV agent.

Our in vitro studies have demonstrated a close correlation between inhibition of HIV-1 protease and anti-HIV activity. However, determination of the therapeutic potential of the protease inhibitor CGP 53437 requires an ultimate assessment in clinical settings.

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