SC-52151, a novel inhibitor of the human immunodeficiency virus protease.

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SC-52151, a Novel Inhibitor of the Human Immunodeficiency Virus Protease


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SC-52151 is a potent, selective, tight-binding human immunodeficiency virus (HIV) protease inhibitor containing the novel (R)-(hydroxyethyl) urea isostere. The mean 50% effective concentration for lytrophic, monocytotropic strains and field isolates of HIV type 1 (HIV-1), HIV-2, and simian immunodeficiency virus is 26 ng/ml (43 nM). The combination of SC-52151 and nucleoside reverse transcriptase inhibitors synergistically inhibited HIV-1 replication without additive toxicity. An extended postantiviral effect correlates with inhibition of gag and gag-pol polyprotein processing. SC-52151 is highly protein bound (>90%) in human plasma, and the level of partitioning into erythrocytes is low. Physiological concentrations of α-1-acid glycoprotein, but not albumin, substantially affect the antiviral potency of SC-52151. The oral bioavailability of [14C]SC-52151 is 17% when it is administered as an elixir to the rat, dog, or monkey. Oxidation of the r-butyl moiety is the major route of biotransformation, and elimination is mainly by biliary excretion. No toxicologically significant effects have been observed in animals. Pharmacokinetic and metabolism studies in multiple animal species predict 20 to 30% systemic bioavailability, an elimination half-life of 1 to 2 h, and a volume of distribution of greater than 3 liters/kg in humans.

The late stage of human immunodeficiency virus (HIV) replication requires a virus-encoded aspartyl protease for maturation of structural proteins and replicative enzyme precursors (12). Inhibition of the protease results in immature, noninfectious virus particles and the cessation of virus propagation. Because of recent advances in peptidomimetic chemistry, novel orally bioavailable inhibitors of the HIV protease have been designed and are undergoing clinical evaluation (18).

SC-52151 represents a unique class of nonnucleoside, urea-based peptidomimetic antiretroviral compounds (8) which are active alone or in combination with reverse transcriptase (RT) inhibitors. SC-52151 is orally bioavailable as an ethanol-containing solution, and the pharmacokinetic properties and metabolism in multiple animal species suggest that an antiviral concentration could be achievable in human plasma.

The antiviral properties of SC-52151 were discussed in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., 4 to 7 October 1994.)

MATERIALS AND METHODS

Chemical description. SC-52151 was synthesized by previously reported methods (8). The structural formula of SC-52151 is given in Fig. 1, and its chemical name is given in the legend to Fig. 1. It is a white, amorphous solid with a molecular weight of 604.75 atomic mass units.

Enzyme assay. HIV protease activity (enzyme concentration, 12 nM) was measured at pH 6.4 with the synthetic heptapeptide AcThrIleNlePhe(p-NO2)GlnArgNH2 by the continuous fluorometric assay developed by Toth and Marshall (22). An increase in fluorescence is generated by the removal of intramolecular fluorescence quenching following specific cleavage of the substrate. Inhibition of the process is detected when cleavage of the peptide is prevented and the rate of increase in fluorescence decreases relative to that in untreated controls. The 50% inhibitory concentrations (IC50) and Ki values were determined by the methods of Cha (4). Similar assays for three mammalian aspartyl proteases, human renin, porcine pepsin, and bovine cathepsin D, were used to determine the selectivity of the inhibitor for the HIV protease. The methods used for the serine, cysteine, and metalloproteases are standard protocols modified where possible for microtiter plate assays (11).

The micro RT assay is an adaptation of several standard RT assays. It was developed to allow small-volume quantitative measurement of HIV RT activity and to facilitate processing of numerous samples (2). The 50% effective concentrations (EC50) were estimated by fitting the four-parameter logistic model (5) to the data by using the SAS nonlinear modeling procedure (PROC NLIN).

Antiviral assays. Antiviral assays were done in 96-well tissue culture plates. MT-2 cells were treated with Polybrene (2 μg/ml) and an 80-μl volume containing 104 cells dispersed in each well. A 100-μl volume of each test article dilution (prepared as a 2% concentration) was added to each of four wells containing 104 cells. HIVINN was diluted in culture medium to a concentration of 5 × 10450% tissue culture infective doses per ml, and a 20-μl volume containing 105 50% tissue culture infective doses of virus was added to three of the wells for each test article concentration. This resulted in a multiplicity of infection of 0.1 for the HIV-infected samples. A 20-μl volume of normal culture medium was added to the remaining well to allow for the evaluation of cytotoxicity. Each plate contained six wells of untreated, uninfected, cell control samples and six wells of untreated, infected, virus control samples. 3′-Azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (ddI) were included as positive control compounds.

The tissue culture plates were incubated at 37°C in a humidified, 5% CO2 atmosphere and were observed microscopically for toxicity and/or a cytopathic effect. At 2 and 5 days after infection, fresh dilutions of each test article were prepared from the frozen stock, and a 20-μl volume of each dilution (prepared as a 10% concentration) was added to the appropriate wells of both infected and uninfected cells after replacement of the culture medium. It was determined in additional experiments that SC-52151 could be added on day 1 and that the cultures could be maintained without replacement of the drug for at least 7 days with no diminution in antiviral activity.

On day 7 postinfection the cells in each well were resuspended and a 50-μl sample of each cell suspension was transferred to a new 96-well plate for use in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (6, 14). A 100-μl volume of fresh RPMI 1640 medium and a 30-μl volume of a 5-mg/ml solution of MTT were added to each 50-μl cell suspension. The cells were incubated at 37°C in 5% CO2 for 4 h. During this incubation MTT was metabolically reduced by living cells, resulting in the production of a colored formazan product. A 50-μl volume of a solution of 20% sodium dodecyl sulfate (SDS) in 0.02 N hydrochloric acid was added to each sample. The samples were incubated overnight, and the absorbance was determined by using a Molecular Devices Vmax microplate reader. This assay detects the drug-induced suppression of viral cytopathic effect, as well as drug cytotoxicity, by measuring the generation of MTT-formazan by surviving cells. An alternative assay used CEMT4 cells and HIV-AEC. Cells infected at multiplicity of infection of 0.001 to 0.010 were added to equal volumes of 2× concentrations of serial threefold drug dilutions. After 3
to 5 days, without further additions of fresh drug culture, the culture supernatants were assayed for RT activity.

Approximately 3 x 10^8 peripheral blood mononuclear cells were activated for 48 h with 2 μg of phytohemagglutinin per ml in RPMI 1640 medium containing 10% fetal bovine serum and interleukin-2 (10 U/ml). Quantitated virus stocks were added to the activated lymphocyte suspension at a multiplicity of infection of 0.005 in RPMI 1640 medium containing 10% fetal bovine serum and interleukin-2 (10 U/ml), and the mixture was incubated for 30 min prior to the addition of SC-52151. These infected cells were added to the test article diluted in complete tissue culture medium from a stock (100 mg/ml) in dimethyl sulfoxide (DMSO) to give 5 x 10^5 cells per well per 200 μl. Infected, untreated cells and cells treated with DMSO alone (0.1%) or either AZT or ddI were used as controls. The assay plates were incubated at 37°C for 7 to 12 days, and culture supernatants were assayed for RT activity. All assays were performed in quadruplicate.

**Postantiviral activity assay.** CEM cells chronically infected with HXB2 (laboratory strain of HIV type 1 [HIV-1]) were added to 6 wells of a 12-well microtiter plate to give 5 x 10^5 cells per well. Half of the wells were treated with test compound at various concentrations, and the same number of uninfected CEM cells were maintained without added compound. Fresh medium with or without test compound was added each day for 3 consecutive days. The cells were then washed twice following the specific treatment to remove residual SC-52151 and were grown in fresh nutrient medium. Virus produced from the chronically infected cells posttreatment was isolated during consecutive 2-h collection periods. The cells were harvested by centrifugation, washed twice in phosphate-buffered saline, and resuspended in 50 μl of 2% Laemml buffer containing 0.125 M Tris (pH 6.8), 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.02% bromophenol blue. Culture supernatant solutions were passed through a 0.22-μm pore-size filter to remove cell debris and were then centrifuged at 10,000 rpm (Beckman 80Ti rotor) for 90 min to concentrate the virus particles. The virus pellet was resuspended to 50 μl of 2% Laemml buffer. The cell or virus suspensions were boiled for 5 min and were then subjected to electrophoretic separation in a SDS-polyacrylamide (10 to 20%) gradient gel. The contents of the gel were then transferred onto nitrocellulose by electroblotting. The HIV-specific bands were visualized by reaction with monoclonal antibodies. In addition, the infectivity of virus produced by the chronically infected cells posttreatment was isolated during consecutive 2-h collection periods. The cells were harvested by centrifugation, washed twice in phosphate-buffered saline, and resuspended in 50 μl of 2% Laemml buffer containing 0.125 M Tris (pH 6.8), 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.02% bromophenol blue. Culture supernatant solutions were passed through a 0.22-μm pore-size filter to remove cell debris and were then centrifuged at 10,000 rpm (Beckman 80Ti rotor) for 90 min to concentrate the virus particles. The virus pellet was resuspended to 50 μl of 2% Laemml buffer. The cell or virus suspensions were boiled for 5 min and were then subjected to electrophoretic separation in a SDS-polyacrylamide (10 to 20%) gradient gel. The contents of the gel were then transferred onto nitrocellulose by electroblotting. The HIV-specific bands were visualized by reaction with monoclonal antibodies. In addition, the infectivity of virus produced by the chronically infected cells posttreatment was isolated during consecutive 2-h collection periods.

**RESULTS**

**Inhibition of HIV protease.** SC-52151 inhibited HIV protease at an IC₅₀ of 3.8 ± 0.6 ng/ml (6.3 ± 0.1 nM) (n = 68) and a Kᵢ of 3 nM. It did not inhibit other aspartyl proteases (e.g., renin, pepsin, or cathepsin D), serine proteases (e.g., leukocyte elastase, pancreatic elastase, plasma kallikrein, chymotrypsin, or trypsin), cysteine proteases (e.g., cathepsin B), or metalloproteases (e.g., angiotensin-converting enzyme), and the IC₅₀ for each of these enzymes was greater than 6.04 μg/ml (10 μM) for a selectivity ratio (IC₅₀ for non-HIV protease/IC₅₀ for HIV protease) in excess of 1,000.

**Antiviral activity.** The antiviral activity of SC-52151 against the HIV_H9 laboratory strain propagated in MT-2 cells was initially determined in nine independent assays. The EC₅₀ of SC-52151 was 0.014 ± 0.011 μg/ml, depending on the dosing schedule (drug addition on day 0 or days 0, 2, and 5) or time (1 h before or 1 h after compound addition) of virus addition. The in vitro antiviral activity of SC-52151 was further evaluated in a rabbit that was infected with SC-52151. The antiviral activity of SC-52151 was 0.003 mg/ml (10 μM), and the EC₅₀ of ddI was 0.17 μg/ml (DD₀₅₀ > 32 μg/ml) in the same antiviral activity assay.

**Antiviral activity against AZT-resistant strains.** The antiviral activity of SC-52151 against a pair of HIV clinical isolates (AZT-resistant HIV-1 [A012]) was obtained from Douglas Richman, AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases), one obtained before the patient was placed on AZT...
therapy (G762.3; AZT susceptible) and the other obtained after the patient was placed on AZT therapy (G691.2; AZT resistant) from the same patient, was determined (Fig. 2). There was no shift in the SC-52151 dose-response curve against the AZT-resistant isolate (EC$_{50}$ for the susceptible and resistant strains, 0.03 m$\mu$g/ml), whereas the AZT dose-response curve showed a significant shift to the right (EC$_{50}$ for the susceptible strain, 0.002 m$\mu$g/ml; EC$_{50}$ for the resistant strain 0.020 m$\mu$g/ml). Several primary clinical isolates of HIV (e.g., 91-793) obtained from patients with AIDS during prolonged treatment with AZT were also resistant to AZT but retained their susceptibilities to SC-52151 (data not shown).

Synergistic antiviral activity of SC-52151 and nucleoside analogs. The combination of SC-52151 and AZT, ddI, didodeoxycytidine (ddC), or stavudine (d4T) was tested in an acute virus infectivity assay against both the AZT-susceptible and AZT-resistant isolates of HIV. The results were evaluated by isobolographic analysis and the general additivity model (19). The results obtained by either method indicated that the antiviral activity of the combination of SC-52151 and AZT was synergistic against the AZT-susceptible virus (Fig. 2). The activities of the combinations of SC-52151 and ddI (Fig. 2), ddC (data not shown), or d4T (data not shown) were synergistic against both the AZT-susceptible and AZT-resistant isolates.

Postantiviral effect. The extent of precursor polyprotein (Pr55$\text{gag}$) processing at the various times posttreatment with SC-52151 was compared with that in untreated samples collected at the equivalent collection times (Fig. 3). Processing of Pr55$\text{gag}$ to p24 and p17 by the HIV protease was not detected until approximately 8 h after drug removal.

The infectivities of the same virus samples are expressed as a percentage of the infectivity of the untreated control samples (Fig. 3). The return of infectious virus production by the chronically infected CEM cells posttreatment with SC-52151 correlated with the extent of polyprotein processing; virus infectivity reached 50% by 26 h posttreatment. Complete recovery of infectious virus production was not observed until later than 72 h posttreatment with SC-52151. The magnitude of the postantiviral effect of SC-52151 depended on the drug concentration and the duration of treatment.

In vitro models of persistent HIV infection. The antiviral activity of SC-52151 was compared with that of AZT in two specialized in vitro models of persistent HIV infection: (i) H9 cells chronically infected with HIV$_{\text{HXB}}$ and (ii) TNF-$\alpha$-treated OM-10.1 cells (7). The first assay measured the reduction in p24 from H9 cells chronically infected with HIV$_{\text{HXB}}$ treated with either SC-52151 or AZT. SC-52151 produced a significant dose-dependent (0.1 to 27 m$\mu$g/ml) inhibition of infectious virus

![Fig. 2. SC-52151 and nucleoside analogs exhibit synergistic antiviral activity. The isobolograms for the antiviral activity of the combination of SC-52151 and AZT against a reference pair of AZT-susceptible (labeled AZT-sensitive) and AZT-resistant isolates are shown. This drug combination has synergistic antiviral activity against the AZT-susceptible isolate and an additive effect against the AZT-resistant isolate. The line of additivity against the AZT-resistant isolate is extrapolated because of the high concentration of AZT alone needed to reach the EC$_{50}$. The activity of the combination of SC-52151 and ddI was synergistic against both the AZT-susceptible and AZT-resistant isolates. Error bars around the EC$_{50}$ determinations for each drug alone are indicated on the x and y axes. One standard deviation around the mean is indicated by the data bars.](http://aac.asm.org/2231)
production (p24 antigen reduction) without cytotoxicity. In contrast, AZT (various concentrations up to 40 μM were used) had no effect. SC-52151 also significantly inhibited p24 antigen production from OM-10.1 cells in a dose-dependent manner, while AZT had no effect.

**Mechanism of action of SC-52151.** Virus produced by chronically infected CEM cells in the presence of 1 to 10 μg SC-52151 per ml was noninfectious, whereas virus produced in untreated cultures was replication competent (Fig. 3). The majority of the virus-related protein in treated cultures was the gag polyprotein precursor. The major capsid protein, p24, and the matrix antigen, p17, were not cleaved from Pr55gag. Electron microscopic examination of the extracellular virus particles produced by the untreated infected CEM cells showed that the particles were morphologically normal (dense cylindrical core), whereas the virus core was absent from the noninfectious virus particles produced by the SC-52151-treated cells (16). The unprocessed precursor polyprotein aberrantly accumulated along the envelope of the virion. The abnormal particles derived from the SC-52151-treated cells retained immunogenic envelope glycoprotein (gp120 and gp41), as well as viral RNA, but they did not regain infectivity when they were incubated in the absence of SC-52151 (data not shown).

**Protein binding.** The binding of SC-52151 to human plasma protein was between 90 and 95%, as determined by a dextran-coated charcoal method, and 90% as determined by using direct binding and filtration (Fig. 4) (11, 24). The nonspecific binding with phosphate buffer was less than 5%. The component of human plasma responsible for the majority of protein binding was investigated by using purified human α1-acid glycoprotein (AAG) and albumin. Twenty to seventy percent of the SC-52151 bound to the AAG at physiological concentrations (0.2 to 2.0 mg/ml) in a dose-dependent manner.

The effect of physiological concentrations of AAG on the antiviral activity of SC-52151 was also measured by using the standard 7-day virus replication assay (Fig. 4 inset). The EC50 of SC-52151 in the presence of 2.0 mg of AAG per ml was consistently increased 18 times the EC50 determined in the absence of AAG. The addition of albumin alone or in combination with AAG had no additional influence on the diminution of the antiviral potency of SC-52151. The effect of the addition of AAG on the intracellular partitioning of [14C]SC-52151 is consistent with these results (21).

**Pharmacokinetics and metabolism of SC-52151.** The half-life of SC-52151 in the rat, dog, and rhesus monkey was less than 1.0 h, and the total body clearance was rapid in these species. The absolute bioavailability of SC-52151 was <1.0% in all animal species unless ethanol was included in the formulation as an elixir. The bioavailability of SC-52151 administered orally to rats and dogs by using the elixir ranged from 5 to 20%, depending on the dose. The absolute bioavailability of total radioactivity after oral administration of [14C]SC-52151 was greater than that observed for the parent compound, suggesting that first-pass metabolism limited the systemic availability of the parent molecule. SC-52151 was extensively distributed into the body, with a volume of distribution much greater than that of the total body water, suggesting that the compound partitioned into all tissues. 14C-radioabeled compound was present in the brain (22.8% brain/plasma, 34.5% as SC-52151) and lymph nodes (198% submaxillary/plasma and 324% mesenteric/plasma). The major metabolites are t-butyl carboxy and t-butyl carboxy isobutyl hydroxy derivatives of SC-52151 which have greatly reduced antiviral activities. The primary route of elimination of the metabolites after intravenous or oral administration is via the feces.

**DISCUSSION**

SC-52151 is a member of a potent class of HIV protease inhibitors incorporating the (hydroxyethyl) urea isostere that...
shows a strong preference for the (R)-hydroxyl isomer, in contrast to, for example, renin inhibitors, which have a preference for the (S)-hydroxyl configuration. SC-52151 also exhibits a unique mode of binding to the enzyme (in which the C-terminal P1’- and P2’-binding groups are inverted) (8). This unique isostere can readily be synthesized from commercially available materials in four steps, and the overall synthesis is nine steps.

SC-52151 has antiviral potency and selectivity similar to those of other HIV protease inhibitors (18). The EC₉⁵ (mean) of SC-52151 against all HIV field isolates tested and the minimal acceptable concentration in plasma targeted in clinical trials is 100 ng/ml. In addition, the antiviral activity of SC-52151 in combination with RT inhibitors is synergistic. Its activity is also synergistic with ddI, ddC, or d4T against AZT-resistant strains of HIV. Additional synergy is observed when SC-52151 is used in triple combination with AZT and the α-glucosidase inhibitor SC-48334, suggesting that inhibition of virus replication at multiple points in its life cycle could be clinically beneficial. In models of chronic or latent HIV infection in which AZT and other nucleoside or nonnucleoside RT inhibitors have no effect, SC-52151 produced a significant dose-dependent inhibition of infectious virus production. In addition, a property unique to SC-52151 and its analogs is the persistence of the antiviral effect after removal of the compound. The postantiviral effect of SC-52151, similar in concept to the well-known postantibiotic effect of penicillin, is dependent on the concentration and the duration of treatment. Unlike the long-lasting antiviral activity observed for the acyclic nucleoside phosphates in animal models of herpesvirus infection, the postantiviral activity of SC-52151 does not correlate with the persistence of drug metabolites intracellularly (15).

One factor that could affect the relationship between the in vitro EC₉⁵ of a drug and the antiviral concentration of that drug required under physiological conditions in vivo is the extent and effect of protein binding. The antiviral activities of several HIV protease inhibitors have been shown to decrease in higher concentrations of human serum or plasma (3). The high binding affinity of SC-52151 for plasma protein, like those of several other HIV protease inhibitors, is probably related to their lipophilicities (10). Bilello et al. (4) have recently shown that AAG but not albumin, both major components of human plasma, can markedly reduce the antiviral activity of the protease inhibitor A77003 and its analogs. AAG is an acute-phase protein whose levels are known to increase after disturbance of homeostasis by infections, inflammation, and injuries. The average concentration of AAG has been reported to be 50 to 65% higher in patients with AIDS than in healthy individuals (17).

The antiviral activities of certain experimental anti-HIV agents, including nucleoside analogs, dextran sulfate, and peptidomimetic-based protease inhibitors (9, 10, 13), are markedly affected by high concentrations of human serum or components of human plasma. Although the structure of a particular drug candidate cannot be used to predict the in vitro plasma protein-binding affinity (20), the reduction in antiviral potency in the presence of physiologically relevant concentrations of plasma or plasma components such as albumin or AAG could be clinically important. SC-52151 can be detected intracellularly in activated peripheral blood mononuclear cells at relevant antiviral concentrations under routine cell culture conditions. It remains to be determined if a reduction in the cellular uptake of SC-52151 correlates with the reduction in antiviral potency and if the postantiviral effect is lost in the presence of AAG.

HIV protease inhibitors are potent antiviral agents with a mechanism of action that is different from yet complementary to those of RT inhibitors. The bridge between in vitro activity and clinical efficacy is being formed at this time by several drug candidates. The accomplishments of preclinical development have been antiviral potency and oral bioavailability of peptidomimetic inhibitors. The challenges remaining include (i) optimization of the dose and the dosing regimen and the importance of attaining a high maximum concentration of drug in plasma and the maintenance of a steady-state concentration at a multiple of the EC₉⁵ during the dosing interval; (ii) determination of the population dynamics of susceptible and resistant virus quasispecies and CD4+ cell number; (iii) determination of the long-term safety of the drug during chronic therapy; (iv) determination of potential drug-drug interactions because of P450 metabolism; (v) determination of the role of protein binding in achieving an effective concentration in plasma or accumulation in tissue; (vi) determination of the level of penetration of the drug into the central nervous system and lymph nodes; (vii) determination of whether combination or sequential therapy with RT inhibitors or multiple protease inhibitors can be used; and (viii) provision of improved potency, decreased clearance, and ease of synthesis to allow reduction in the cost of long-term treatment.

REFERENCES