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Antiviral Efficacy In Vivo of the Anti-Human Immunodeficiency Virus Bicyclam SDZ SID 791 (JM 3100), an Inhibitor of Infectious Cell Entry

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SID 791, a bicyclam inhibiting human immunodeficiency virus (HIV) replication in vitro by blocking virus entry into cells, is an effective inhibitor of virus production and of depletion of human $CD4^+$ T cells in HIV type 1-infected SCID-hu Thy/Liv mice. Steady levels of 100 ng of SID 791 or higher per ml in plasma resulted in statistically significant inhibition of p24 antigen formation. Daily injections of SID 791 caused a dose-dependent decrease in viremia, and this inhibition could be potentiated by coadministration of zidovudine or didanose. The present study suggests that SID 791 alone or in combination with licensed antiviral agents may decrease the virus load in HIV-infected patients and, by extension, that the infectious cell entry step is a valid target for antiviral chemotherapy of HIV disease. The SCID-hu Thy/Liv model in effect provides a rapid means of assessing the potential of compounds with novel modes of antiviral action, as well as the potential of antiviral drug combinations.

Bicyclams are a new class of anti-human immunodeficiency virus (anti-HIV) agents, selective for HIV type 1 (HIV-1) and HIV-2 (4, 6, 7). The compounds inhibit an early step in the life cycle of HIV, presumably a fusion step associated with virus entry into cells (8, 9). Within the class of bicyclams, which are macrocyclic molecules containing two linked monocyclam (1,4,8,11-tetraazacyclotetradecane) units, potent anti-HIV activity and low levels of cytotoxicity are highly dependent on the nature of the linker connecting the cyclam moieties through nitrogen (4, 13). When the cyclam rings are linked via a phenylenebis(methylene) unit attached to the nitrogens, the result is one of the most potent and selective anti-HIV compounds, SID 791, also referred to as JM 3100 (4, 6). SID 791 inhibits replication of several clinical HIV-1 isolates in primary T cells and monocytes ≥99.9% at concentrations of 12 to 36 nM (10 to 30 ng/ml) without being cytotoxic at 400 μM (6). To determine whether this mechanistically novel agent with potent antiviral activity in vitro shows antiviral efficacy in vivo, we investigated the inhibition of HIV-1 replication in intrathymically infected SCID-hu Thy/Liv mice.

The SCID-hu mouse is a heterochimeric small-animal model in which human lymphoid organs are surgically transplanted into the immunodeficient C.B-17 *scid/scid* mouse. When fetal liver is implanted together with fetal thymus (SCID-hu Thy/Liv), long-term hematopoiesis with human blood cells occurs, and the grafts remain viable for up to 12 months following implantation (15, 16, 19). Intrathymic injection of HIV leads to infection in virtually 100% of animals in a time-dependent manner. Viral infection can be detected by DNA PCR of thymocytes as early as 3 days postinoculation. HIV p24 protein becomes measurable by enzyme-linked immunosorbent assay (ELISA) at about 5 days postinoculation, and the levels increase until human thymocytes from all SCID-hu mice are positive for HIV DNA and for HIV p24 and are positive by viral coculture by days 10 to 14 postinfection (17). The time course of the infection is both virus isolate and titer specific. As infection proceeds past day 14, the $CD4^+$ cells and the $CD4^+$ $CD8^+$ cells in the thymus begin to die (1, 3, 14, 15, 18). This pathogenic effect of HIV becomes more apparent at 3 to 4 weeks postinoculation, when a significant decrease in the size of the $CD4^+$ $CD8^+$ population and an inversion of the CD4/CD8 ratio can be detected. The model can be used to study the efficacies of antiviral agents by using as endpoints (readouts) p24 production and analysis of human $CD4^+$ and $CD8^+$ cells. A validation study with zidovudine (AZT) and didanosine (DDI) showed a statistically significant reduction in p24 antigen levels and suppression of thymocyte depletion at doses comparable to those known to be efficacious in humans (17).

In rodents, SID 791 has a negligible first-pass effect and is highly bioavailable (>85%) when it is administered subcutaneously (10). At nontoxic doses, the levels of SID 791 in plasma and serum in excess of 90% inhibitory concentrations can be readily obtained. Furthermore, in rabbit plasma, SID 791 occurs in an antivirally active form (20), and mouse serum (10%) does not interfere with the antiviral activity of SID 791. The experiments reported here show that SID 791 can inhibit HIV replication in the SCID-hu Thy/Liv model. This indicates that targets other than reverse transcriptase or HIV protease are suitable for antiviral chemotherapy of HIV disease.

MATERIALS AND METHODS

Antiviral compounds. The bicyclam SID 791 was synthesized as described by Bridger et al. (4). For studies with animals, the compound was dissolved in water and the pH was brought to pH 7.3 by the addition of $NaHCO_3$. At room temperature the compound is stable under these conditions for at least 2 weeks. Dose levels (in milligrams per kilogram of body weight) refer to the doses of the salt form of the compound (SID 791 is an octahydrochloride salt). AZT was obtained from Sigma; DDI was obtained from the repository of the National Institutes of Health. AZT solutions were prepared in water and were then filtered through a 0.2-μm-pore-size filter, divided into aliquots, and stored at 4°C until they were used. Solutions of DDI were prepared in filtered (pore size, 0.2 μm) water adjusted to approximately pH 9.5; aliquots were stored at 4°C until use.

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Levels of SID 791 in plasma. SID 791 concentrations in plasma were determined by using two specific high-performance liquid chromatography (HPLC) assays. Usually, the samples were analyzed by a method with UV detection of a copper complex of SID 791 (20). Additionally, a method with electrochemical detection was used for samples with low SID 791 concentrations. To extract the bicyclam, plasma samples (200 μl) were mixed with 50 μl of a saturated NaOH solution and were extracted with 1 ml water-saturated *t*-butylmethylether in a 2.1-ml polypropylene centrifuge vial for 3 h on a shaker. A total of 850 μl of the ether phase was transferred to a second 2.1-ml vial and was extracted for 1 h under frequent shaking with 850 μl of 2% trifluoroacetic acid (TFA). The TFA phase (800 μl) was transferred to a 1.2-ml centrifuge vial and was dried down in a Speed Vac centrifuge. For UV detection, the residue was dissolved in 50 μl of water and 10 μl of 0.25 M copper(II) acetate. The solution was heated at 65°C for 15 min to convert the compound into its stable and strongly UV-absorbing copper complex (20). Aliquots of 50 μl were injected into the HPLC system. For electrochemical detection, the extracted residues were dissolved in 20 μl of 0.02% TFA.

The HPLC system for UV detection has been described earlier (20). The detection limit of SID 791 was about 80 ng/ml of plasma.

The HPLC system for electrochemical detection consisted of the following components: PLRP-S 250-by-4 mm column (Polymer Laboratories Ltd., Shropshire, United Kingdom), a Waters model 590 pump, a Waters model U6K injector, a Metrohm model 656 amperometric detector /641 VA detector with a 1-μl cell with glassy carbon working and auxilliary electrode, and a Ag/AgCl reference electrode. The mobile phase was a mixture of 700 ml of water (HPLC quality), 360 ml of acetonitrile, 5 ml of tetrabutylammonium hydroxide, 3 ml of sodium lauryl sulfate (2%; wt/vol), and 50 mg of sodium EDTA. The solvent was filtered through 0.2-μm-pore-size, membranes and degassed by ultrasound and He sparging. Analytical runs of 12 min were made at a flow rate of 0.8 ml/min and ambient temperature, a sensitivity of 1 nA, and an oxidation potential of +700 mV.

SID 791 eluted as one peak after 7.5 min; the peak area was used for quantitation. Calibration runs were made for each analytical series with external standards in the range of 20 to 200 ng/ml of plasma extracted from spiked 200-μl plasma samples as described above. The calibration curves were linear, with a regression coefficient of 0.999. The detection limit of SID 791 was about 10 ng/ml of plasma.

Virus. Titered virus stocks were prepared as described earlier (17). The clinical isolate EW was derived from an HIV-1-seropositive patient. Seed stocks were obtained after cocultivation of patient-derived, phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells with PHA-activated peripheral blood mononuclear cells from healthy donors. Seed stocks of the molecular clone NL4-3 (repository of the National Institutes of Health) were obtained by electroporation of 25 μg of HIV DNA per 5×10^6 cells (Bio-Rad Gene Pulser) at 960 μFD and 280 V.

The 50% tissue culture infective dose of HIV-1 was determined in PHA-activated peripheral blood mononuclear cells by using 5- to 10-fold serial dilutions of virus in 4 replicate wells of a 96-well plate. The 50% tissue culture infective dose is expressed as the reciprocal of the dilution at which 50% of the wells contained detectable p24 (17).

HIV-1 infection and treatment of SCID-hu Thy/Liv mice. The SCID-hu Thy-Liv mice were constructed as described by Namikawa et al. (16). The animals were inoculated by direct intrathymic injection of small volumes of standardized viral stocks, typically 10^3 50% tissue culture infective doses in 25 μl (17). Unless indicated otherwise, treatment was started 1 day before infection; a second dose of antiviral agent was given 1 h before infection. Treatment was done by subcutaneous injection or via subcutaneously implanted Alzet minipumps. The Alzet pumps (model 2002) were primed by incubation in 0.86% NaCl for 14 h at 37°C and were implanted 24 h before infection. SID 791 was dissolved in 0.1 M $NaHCO_3$, adjusted to pH 7.0, and delivered at a rate of 0.5 μl/h.

Analysis of antiviral efficacy. Unless indicated otherwise, the mice were killed at day 14 postinfection. The transplanted human Thy/Liv implants were removed, and a single-cell suspension was prepared as described previously (17). For quantitation of HIV-1 p24 within cells, 5×10^6 cells were lysed (17), and the amount of p24 was measured by a quantitative ELISA (Dupont) by using HIV-1 HXB2-infected H9 cells to generate standard curves. Results are recorded as the number of picograms of p24 per 10^6 cells. The fluorescence-activated cell sorter (FACS) analysis for thymocyte depletion used 10^6 pelleted cells suspended in 80 μl of a monoclonal antibody cocktail containing CD4-fluorescein isothiocyanate, CD8-phycoerythrin, and CD3-Tandem Color (Becton Dickinson) with isotype controls for each antibody mixture. Analysis was as described by Rabin et al. (17).

In all experiments, five to eight positive control mice were infected with HIV-1 but were not treated with antiviral compounds and one to five negative control mice were not infected with HIV-1 and were not treated. In the experimental groups, five to eight mice per group were infected and treated with various doses of antiviral compound. The p24 results for the drug-treated groups in a given experiment are expressed as the mean amount of p24 produced per 10^6 cells in the infected, untreated group. These results are expressed as the mean ± standard error for a given group. Statistical analyses were carried out by a nonparametric method by using the Mann-Whitney U test.

TABLE 1. Levels of SID 791 in plasma of mice following administration of a 10-mg/kg s.c. dose

Time (h)	Mean concn in plasma (μg/ml)[a]
0.166	21.00 ± 1.61
0.5	12.67 ± 2.15
1	4.13 ± 1.97
2	0.56 ± 0.24
3	0.21 ± 0.08
5	0.18 ± 0.09
8	0.10 ± 0.01

[a] The levels in plasma given here shown are means ± standard deviations obtained for three animals per sampling time.

RESULTS

Levels of SID 791 in plasma of BALB/c-mice following drug administration by s.c. injection or via Alzet minipumps. Prior to determining the efficacy of SID 791 in infected SCID-hu Thy/Liv mice, the levels of the bicyclam in the plasma of mice were determined following drug administration by the two methods used: subcutaneous (s.c.) injection and slow release by means of s.c.-implanted Alzet mini-pumps (dose levels refer to the doses of the salt form of the compound [SID 791]). The reasons for using the s.c. route of drug administration are outlined in the Introduction. It ensured reproducible levels of drug in plasma. Administration of the drug, dissolved in buffer, by the oral route led to highly variable drug levels (10). Hence, this route of administration of the drug in buffer was not suitable for the present study. Table 1 provides the levels of SID 791 in plasma after the administration of a single s.c. dose of 10 mg/kg. Peak levels in plasma were 21 μg/ml at 0.2 h after dosing (first time point). The levels in plasma fell rapidly to 0.2 μg/ml at 3 h postdosing. The area under the concentration-time curve from time zero to infinity for the s.c. dose of 10 mg/kg was 15 μg · h/ml. Alzet osmotic minipumps (model 2002) filled with 30, 10, 3, or 1 mg of SID 791 per ml of sterile 0.1 M $NaHCO_3$ were prerun in 0.86% NaCl at 37°C for 14 h before implantation. At days 1, 2, 5, and 10 after implantation, the levels of SID 791 were determined in plasma (three animals per sampling time and dose group). The results (Table 2) show fairly constant drug levels over the 10-day period.

Effects of daily s.c. doses of SID 791 on virus production. SCID-hu Thy/Liv mice were infected intrathymically with HIV-1 EW 1 day after the initiation of treatment and 1 h after the administration of the second dose of the bicyclam. SID 791 was given s.c., once a day, changing injection sites daily, at dosages of 0.3, 1, 3, and 10 mg/kg/day for 14 days. After the 2-week treatment, the amount of viral p24 in the thymocytes from the human implant was determined by ELISA and was normalized to obtain the amount per 10^6 cells. The results (Table 3) show a dose-dependent decrease in p24 levels, with a 50% effective dosage of approximately 1 mg/kg/day ($P = 0.028$).

Here, as in other studies (17), we observed that the capacity of the Thy/Liv implants to support replication was dependent on the organ donor (data not shown). However, when expressed as a percentage of the value for the control, the variation in absolute p24 levels was normalized. Thus, for a dosage of 10 mg of SID 791 per kg/day and infection by HIV-1 EW, the mean ± standard error value for p24 production was 24.5% ± 4.9% ($n = 11$) of the level in untreated, infected animals. SID 791 was also active in the SCID-hu Thy/Liv model when HIV-1 NL4-3 was used (once-daily injections for 14 days). A statistically significant 89% inhibition of p24 production was

TABLE 2. Levels of SID 791 plasma of mice following s.c. implantation of an osmotic drug delivery pump

Sampling time (day)	SID 791 concn in pump (mg/ml)	Mean concn in plasma (μg/ml)[a]
1	30	0.70 ± 0.09
	10	0.12 ± 0.07[b]
	3	0.08 ± 0.01[b]
	1	<0.07[c]
2	30	0.69 ± 0.44
	10	0.21 ± 0.06
	3	0.06 ± 0.02[b]
	1	0.02 ± 0.01
5	30	0.72 ± 0.17
	10	0.20 ± 0.09
	3	0.09 ± 0.09
	1	0.05 ± 0.04[d]
10	30	0.66 ± 0.30
	10	0.30 ± 0.20
	3	0.06 ± 0.04
	1	0.06[e]

[a] The levels in plasma given here are average values obtained for three animals per sample time and dose, unless indicated otherwise.
[b] Data are values from two animals; one value was below UV detection limit of 0.07 μg/ml.
[c] Values from two animals were below UV detection limit.
[d] Data are values from two animals.
[e] Data are values from one animal only.

obtained at 10 mg of SID 791 per kg/day ($P = 0.01$ for the means for the treated groups compared with those for the untreated groups [data not shown]).

Treatment of HIV-1 EW-infected SCID-hu Thy/Liv mice with 10 mg of SID 791 per kg/day could be postponed to 5 days postinfection and still result in inhibition (81% relative to that in untreated mice) of p24 production (data not shown). Postponement to 7 days postinfection decreased inhibition to 44% of that in untreated mice (P was not significant).

Effects of the frequency of dosing of SID 791 on virus production. SCID-hu Thy/Liv mice were infected as described above, and SID 791 was administered at 10 mg/kg, either once daily (as above) or every other day. The amount of p24 in human thymocytes was determined after 2 weeks of treatment. Once-daily administration resulted in 65% inhibition of p24 production in this experiment ($P = 0.055$), whereas administration of 10 mg/kg/day every other day had no effect ($P = 0.523$). The amount of p24 produced following once-daily administration of SID 791 was significantly different from that following dosing schedule of every other day ($P = 0.006$).

TABLE 3. Effect of SID 791 on p24 production in HIV EW-infected SCID-hu Thy/Liv mice[a]

Dosage (mg/kg/day)	p24 level (pg/10^6 cells)	P value
0	394 ± 107	
0.3	280 ± 48	0.61
1	161 ± 46	0.028
3	122 ± 33	0.010
10	73 ± 15	0.003

[a] Groups of eight mice each were treated with SID 791 s.c. once daily for 14 days at the indicated doses. The p24 values are means ± standard errors. Statistical analysis was done by the Kruskal-Wallis one-way analysis of variance; the dependent variable was p24. The P values are derived from comparisons of means for treated groups relative to those for the untreated (control) groups by the Mann-Whitney U test; nonparametric analysis was used.

TABLE 4. Effects of SID 791 on the CD4/CD8 ratio and p24 production in HIV EW-infected SCID-hu Thy/Liv mice[a]

Day post-infection	CD4/CD8 ratio			p24 level (pg/10^6 cells)	
	Uninfected, treated animals	Infected, untreated animals	Infected, treated animals	Infected, untreated animals	Infected, treated animals
0	1.9[b]			0	0
14	2.0 ± 0.1	1.6 ± 0.2	2.0 ± 0.1	129 ± 46	12 ± 9
21	1.8 ± 0.2	0.7 ± 0.1	1.3 ± 0.1	261 ± 88	106 ± 28
28	1.0 ± 0.2	0.6 ± 0.1	1.1 ± 0.1	367 ± 88	191 ± 44

[a] SID 791 (10 mg/kg/day b.i.d.) was given s.c. The CD4/CD8 ratio was determined by FACS analysis. Values ± are means ± standard errors.
[b] The decrease in the CD4/CD8 ratio in uninfected, treated animals was also observed in uninfected, untreated animals.

In a different experiment, the effects of 10 mg of SID 791 per kg/day given either once daily or twice daily (b.i.d.) were compared. The difference in inhibition of p24 production (80 and 72% for the daily and the b.i.d. schedules, respectively) was not statistically significant. A dosage of 20 mg/kg/day b.i.d. in this experiment resulted in 97% inhibition of p24 production ($P = 0.001$ relative to that in untreated control mice and $P = 0.071$ relative to that in mice given a dosage of 10 mg/kg/day b.i.d.). Thus, two daily injections (12 h apart) of 5 mg of SID 791 per kg are as efficacious as one injection of 10 mg/kg; two daily injections of 10 mg/kg give somewhat better protection than a single daily dose of 10 mg/kg.

Effects of SID 791 on the CD4/CD8 ratio. HIV infection of SCID-hu (Thy/Liv) mice results in a decrease of the percentage $CD4^+$ $CD8^+$ thymocytes and an inversion of the CD4/CD8 ratio by 3 to 4 weeks after inoculation (3, 17). In fact, p24 production peaks at the time (ca. 2 weeks postinfection) when the decrease in the percentage of $CD4^+$ cells and $CD4^+$ $CD8^+$ cells begins. As the infection process continues, p24 values become increasingly variable; at 3 to 4 weeks postinfection the thymus contains a mixture of infected cells and dead (dying) cells, and the p24 levels decrease as the target cells are destroyed. To investigate the effects of SID 791 on both p24 production and the CD4/CD8 ratio, analyses were performed at 2, 3, and 4 weeks after inoculation.

The results presented in Table 4 indicate that in parallel with an inhibition of p24 production, SID 791 prevented the decrease in the CD4/CD8 ratio. Thus, 90 and 59% inhibition of p24 production was seen in SID 791-treated animals relative to that in untreated control animals at 2 and 3 weeks after inoculation, respectively ($P = 0.016$ and 0.150, respectively). The FACS analysis for the CD4/CD8 ratio indicates protection by treatment with SID 791 relative to that by no treatment at the 2-, 3-, and 4-week time points ($P = 0.150$, 0.004, and 0.004, respectively).

Effects of SID 791 in combination with DDI or AZT on virus production. SCID-hu Thy/Liv mice were infected with HIV-1 EW and treated with SID 791 (5 mg/kg/day b.i.d. given s.c.), AZT (5 mg/kg/day b.i.d. given orally), or DDI (1 mg/kg/day once daily given intraperitoneally) or with a combination of SID 791 plus AZT or a combination of SID 791 plus DDI at the indicated dosages. The amount of p24 was determined 14 days after infection.

When administered as single agents at the dosages used, SID 791, AZT, and DDI had limited, statistically nonsignificant effects on viral p24 production, i.e., 37, 18, and 40% inhibition,

TABLE 5. Effect of SID 791 on p24 production when administered via Alzet minipumps[a]

SID 791		p24 level ($pg/10^6$ cells)	*P* value (treated to untreated animals)	SID 791 levels in plasma ($\mu g/ml$)
Dose (mg/ml) in pump	Dosage (mg/kg/day)			
30	11.0	3.2 ± 1.2	0.0027	0.389 ± 0.378
10	3.8	20.8 ± 5.9	0.0027	0.117 ± 0.047
3	1.1	51.0 ± 10.9	0.004	<0.040[b]
0	0	137.2 ± 20.8		

[a] Groups of seven mice each were treated with SID 791 via implanted Alzet minipumps. The p24 values are means ± standard errors. Statistical analysis was done by the Kruskal-Wallis one-way analysis of variance; the dependent variable was p24. The *P* values were derived from comparisons of means for treated groups relative to those for the untreated (control) groups by the Mann-Whitney U test; nonparametric analysis was used. Drug levels were determined at day 14 and are the mean ± standard deviations for seven mice.

[b] In two of seven animals drug levels were below the detection level (10 ng/ml); the average drug level in the remaining five animals was 0.040 ± 0.0014 μg/ml.

respectively, compared with the levels in untreated animals ($P = 0.361$, >0.999, and 0.262, respectively). The combination of SID 791 plus AZT resulted in 74% inhibition relative to the level in untreated control animals ($P = 0.028$). The combination of SID 791 plus DDI resulted in 89% inhibition relative to the level in untreated control animals ($P = 0.016$). When the p24 levels in the thymocytes from animals treated with combinations of drugs were compared with those in the thymocytes from animals treated with single agents, a statistically significant difference was observed for the SID 791-DDI combination relative to that for SID 791 alone ($P = 0.037$) and relative to that for DDI alone ($P = 0.037$). No significant differences were observed for the SID 791-AZT combination relative to that for SID 791 alone ($P = 0.251$) or AZT alone ($P = 0.100$).

Effects of continuous administration of SID 791 on virus production. After the administration of 10 mg of SID 791 per kg to BALB/c mice, plasma drug levels were as low as 0.1 to 0.2 μg/ml at 3 h postdosing. Yet, single doses of 10 mg/kg are effective in reducing the virus load in SCID-hu Thy/Liv mice. The administration of SID 791 in a slow-release form could avoid high peak levels in plasma while maintaining constant levels of drug sufficient to suppress virus replication. We used Alzet osmotic minipumps (model 2002) to achieve this. The pumps were filled with SID 791 in neutral aqueous solutions at 30, 10, or 3 mg/ml and were implanted into the mice 24 h prior to infection with HIV-1 EW. After 2 weeks the amounts of p24 and the levels of SID 791 in plasma were determined. Table 5 provides the results of a representative experiment and demonstrates a clear dose-dependent reduction in the level of viremia by this route of administration, with dosages of ≥1.1 mg/kg/day giving statistically significant reductions in virus load. A continuous drug concentration in plasma of approximately 100 ng/ml is sufficient to reduce the virus load significantly. The initiation of treatment by the slow release of SID 791 (11 mg/kg/day) could be postponed to 5 days postinfection and still result in 94% inhibition of p24 production relative to that in untreated animals. Initiation of treatment at 7 days postinfection caused 62% inhibition (*P* was not significant).

DISCUSSION

In SCID-hu mice transplanted with a human fetal thymus-liver conjoint organ, HIV-1 replicates to high titers when it is inoculated intrathymically. Over time, depletion of human $CD4^+$ thymocytes occurs, causing an inversion of the CD4/CD8 ratio. We have shown here that an antiviral bicyclam, SID 791, can inhibit HIV-1 replication and the associated immune cell destruction. This implies that the virus entry step into cells, the step inhibited by SID 791, is a valid target for antiviral chemotherapy of HIV disease. To date, antiviral efficacy had been shown only with inhibitors of HIV reverse transcriptase and HIV protease.

The slow release of SID 791 from subcutaneously implanted minipumps, which results in steady levels in plasma of approximately 100 ng of SID 791 per ml, is effective in giving a statistically significant 85% inhibition of virus replication in the SCID-hu Thy/Liv mouse model. These levels in plasma are approximately 10 times higher than the concentration giving ≥99.9% inhibition of virus replication in lymphocytes or monocytes (6). Antiviral efficacy is maintained when treatment is initiated 5 days after virus inoculation. Furthermore, the antiviral efficacy of SID 791 is dose dependent: increasing the levels of SID 791 in plasma increases the inhibition of virus replication. Because SID 791 has a charge of +4 at physiological pH, it is unlikely to enter cells rapidly, if at all. Presumably, therefore, the levels of this agent, which targets gp120 of the HIV envelope (8), in plasma can predict antiviral efficacy. Indeed, earlier we had already shown that in rabbits dosed with SID 791, the levels of SID 791, which binds strongly to plasma proteins (10), in plasma are identical whether the drug levels are assayed by measuring the antiviral activity or the drug in serum samples or by HPLC (20).

Earlier studies (6, 7) suggested that bicyclams such as SID 791 inhibit an event defined experimentally as uncoating. In fact, those studies were not yet able to differentiate whether the drug targeted a post-CD4-binding fusion event or the uncoating of viral RNA. Newer studies have conclusively shown that SID 791 inhibits fusion and targets the gp120 envelope glycoprotein (8, 9). In other virus systems, such as herpes simplex virus infections (2), intracellular virus metabolism offers the preferred targets for antiviral chemotherapy. Because HIV infection of the fetal thymus-liver conjoint organ in SCID-hu Thy/Liv mice organ has to date been shown to be an accurate model of HIV pathogenesis (1, 3, 14, 18), it appears that the infectious cell entry step is a validated target for antiviral chemotherapy of HIV disease.

Our finding that SID 791 shows antiviral efficacy whether it is administered by slow release or by s.c. injections implies that the peak levels of the drug obtained after s.c. injection are not required for efficacy. Steady, high levels of the drug (high levels relative to the 90% inhibitory concentration) may diminish the risk of outgrowth of resistant virus and also decrease the unwanted side effects of the bicyclam. Drug levels cycling through peaks and troughs may favor the outgrowth of drug-resistant virus strains and can be avoided by developing a service form of SID 791 enabling slow release from the sites of s.c. administration without losing antiviral efficacy. In addition, the inherent rate of development of resistance to SID 791 is very low, as assessed by repeated passaging of virus in cell culture while allowing minimal virus replication, presumably because of the requirement for multiple mutations to obtain resistance (8). Taken together, these findings suggest the possibility that SID 791 has sustained antiviral efficacy in HIV-infected patients.

Nevirapine, given in two daily oral doses of 12.5 mg/kg, gives statistically significant inhibition of p24 production in HIV-1-infected SCID-hu Thy/Liv mice (17). In humans, a single oral dose of 12.5 mg (ca. 0.2 mg/kg) is sufficient to decrease p24 levels in serum (13, 14) at levels of nevirapine in plasma similar (5, 14) to those in the plasma of mice (17) after the administration of a dose of 10 mg/kg. Although nevirapine acts intra-

cellularly, inhibiting HIV reverse transcriptase, it does not, like SID 791, need to be metabolized to exert its antiviral effects. Thus, a rough estimate of the dose in humans required to decrease p24 levels in serum is 1/100th of the dose giving statistically significant inhibition of p24 production in SCID-hu Thy/Liv mice or, for SID 791, 0.01 to 0.1 mg/kg. For sustained antiviral efficacy, however, escape of drug-resistant virus strains must be prevented; as indicated above, steady, high levels of SID 791 in plasma may be able to achieve this.

The SCID-hu (Thy/Liv) model also appears to be suitable for demonstration of the efficacies of drug combinations. We have shown that both AZT and DDI potentiate the antiviral efficacy of SID 791 in vivo. Furthermore, a statistical analysis showed the superiority of the SID 791-DDI combination over either agent alone. We therefore believe that the SCID-hu Thy/Liv model of HIV infection is a useful small-animal model for the rapid assessment of the antiviral efficacy of a particular drug combination.

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