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Antiviral Research 27 (1995) 85–97

Antiviral Research

Efficacy of AZT therapy in reducing p24 antigen burden in a modified SCID mouse model of HIV infection.

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Received 1 November 1994; accepted 22 November 1994

Abstract

A modified severe combined immunodeficient (SCID) mouse model of HIV infection which utilized multiple reconstitutions with human lymphocytes and a large inoculum of HIV was investigated. This mouse model yielded splenic HIV p24 antigen concentrations detectable by standard clinical means. The concentration of p24 exceeded 600 pg/g of spleen through 4 weeks postinfection. A 1-week course of AZT therapy initiated after infection produced a dose responsive reduction in p24 antigen burden. Up to a 95% reduction in p24 antigen burden was observed following AZT therapy at 50 mg/kg/day, while AZT therapy at 5 and 0.5 mg/kg/day produced 52 and 18% reductions. In vitro and pharmacokinetic evaluations correlated potency and tissue concentrations of AZT with treatment efficacy. Active HIV replication in the SCID mice was suggested by both the recovery of viable virus from SCID spleens, and by the efficacy of a brief course of AZT therapy. This SCID mouse model of HIV infection was more quantitative than previous mouse models that utilize PCR-based techniques for detection of HIV. The high HIV burden in this SCID mouse model allowed reductions in p24 concentration to be monitored in response to AZT therapy. A dose response to AZT therapy was demonstrated, even when the first dose was administered after infection. This result suggests greater sensitivity than in previous models in which pretreament with AZT was required to produce a protective response. This SCID mouse model may be useful for determining efficacy of experimental HIV therapeutics prior to clinical use. An effective animal model could result in a reduction in cost and more rapid development of effective HIV therapeutics.

Keywords: HIV; Severe combined immunodeficient (SCID) mouse; Mouse model; AZT

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SSDI 0166-3542(94)00079-4

1. Introduction

The failure of experimental HIV therapeutics in the clinic has resulted in increased cost and delays in drug development. However, there is no established in vivo method to test experimental HIV therapeutics for efficacy prior to clinical use. Clinical trials are cumbersome when used as the initial test for in vivo efficacy against HIV because relatively long periods are required for approval, initiation and evaluation. HIV patients may be compromised as test subjects due to opportunistic infections requiring other drug therapies, and placebo controls are often not ethical. The long incubation period of HIV infection and the variety of clinical manifestations leads to difficulty in obtaining matched test subjects.

Current animal models of HIV infection are technically difficult and unwieldy for testing of HIV therapeutics. The inability of HIV virus to directly infect small laboratory animals has been a major obstacle. The approach of using severe combined immunodeficient (SCID) mice reconstituted with suitable human target cells (Mosier et al., 1988; Mosier, 1990) has proved successful for replication of HIV virus in mice (Namikawa et al., 1988; McCune et al., 1990a; Mosier et al., 1991). The SCID mouse models of infection have yielded HIV viral burdens which were detected by non-quantitative molecular biological techniques, most commonly the polymerase chain reaction (PCR) technique. HIV has been detected in human xenograft implants in SCID mice (Namikana et al., 1988) and in spleens of reconstituted SCID mice (Mosier et al., 1991), as well as in xenograft human tumor implants in nude mice (Wetherall, 1990). Infectious HIV has also been recovered from transgenic mice developed by Leonard et al. (1989). The infected mice developed no pathology as a result of HIV infection.

There is need for an effective small animal model of HIV infection to facilitate rapid and reliable testing of therapeutics. HIV drug evaluations often utilize an enzymatic or biochemical assay as the first screen, followed by a whole cell assay for inhibition of HIV replication. Potential clinical efficacy is projected by pharmacokinetic evaluation matched to in vitro potency of test compounds. Toxicity evaluation is then used for estimation of therapeutic margins. In vitro systems are also used to model in vivo considerations, such as the effect of serum binding and potential synergy of drug combinations. Some recent in vitro models of human pharmacokinetics are quite detailed, allowing better predictions of drug efficacy (Bilello et al., 1994). However, there is no requirement for demonstration of in vivo efficacy of HIV compounds prior to clinical use.

Early clinical testing of HIV compounds is encouraged by the lethal and transmissible nature of HIV infection and the lack of curative therapy. However, the absence of predictive preclinical evaluation may lead to clinical testing of ineffective compounds, which ultimately increases cost and slows development of effective HIV therapeutics.

The SCID/HIV mouse model described in this report may be of utility in HIV drug development programs. High burdens of replicating HIV were produced in SCID mice. Viral burden was quantitated by using clinical HIV-1 p24 antigen detection techniques. High p24 antigen levels were detected in spleen homogenates of reconstituted SCID mice up to 4 weeks postinoculation. This allowed for a period for therapy. AZT treatment was effective in reducing both the viral burden of infected mice and the

proportion of mice with detectable viral burden. However, the trial-to-trial variability of HIV p24 antigen burden in untreated mice (413–1566 pg p24/g spleen) complicates analysis. Also, the biohazard factors of HIV inoculation and the lack of animal pathology render this model less than ideal for testing of experimental therapeutics.

2. Materials and methods

2.1. *In vitro* IC_{50} *determination*

A standard CPE reduction assay was used for determination of the potency of AZT versus HIV-III_B. This assay was based on inhibition of HIV-induced cytopathic effect on MT-4 cells as detected by measuring cell viability following a 5-day incubation with virus and drug (Pauwels et al., 1988). Cell viability was measured by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, MO) by viable cells. Reduced MTT was blue in color and was detected spectrophotometrically at 540 nm. The absorbance at 690 nm (A_{690}) was subtracted from A_{540} to correct for non-specific absorption. The percent protection produced by AZT was calculated as in Pauwels et al. (1988) based on optical densities of cells plus drug plus HIV, cells plus HIV, and cells alone. The drug concentration to produce 50% protection was calculated by median effect equation (Chou and Rideout, 1976). 3′-Azido-2′,3′-dideoxythymidine (AZT) was purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of AZT were made in phosphate-buffered saline (PBS, pH = 6.8) diluted in RPMI 1640 medium at $10 \times$ final concentration. HIV-III_B was obtained from Dr. R.C. Gallo and was cultured on MT-4 cells as described (Gallo et al., 1984).

2.2. *In vivo trials*

All trials were conducted in a biological safety level 3 facility. Female BALB/C BYJ/IMD SCID mice (SCID), 4–5 weeks of age, were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mice were housed in sterilized cages and were fed sterile food and water. Mice were reconstituted by intraperitoneal injection of 1×10^8 human lymphocytes 7 and 5 days before HIV infection, and 2 h and 5 days postinfection. Human lymphocytes were isolated from buffy coats purchased from Lifesource® (Chicago, IL). Lymphocytes were isolated from approximately 25 ml of buffy coat suspension by centrifugation through Histopaque® solution (Sigma).

HIV-III_B was grown in 1-l flasks containing MT-4 cells as described by Gallo et al. (1984). HIV was collected when the cultures were estimated to be at maximal viral load (3 days postpropagation). The inoculum for each group of 10 mice was prepared from 50-ml aliquots of culture. The aliquots were resuspended, centrifuged at 1000 *g* for 10 min and the supernatant was discarded to yield a total volume of 5.5 ml. Cell pellets were resuspended in the remaining supernatant yielding a concentration of approximately 1×10^9 cells and 10–50 mg p24 per ml in 5.5 ml. As control, an inoculum of killed HIV was prepared in the same way as the standard inoculum, with the addition of

a 1-h incubation in 0.5% Triton X-100 (Sigma). An aliquot was taken for p24 antigen assay to determine the relative viral load of the culture.

Prior to inoculations, mice were placed under anesthesia by i.p. injection of 1.43 mg Nembutanol® (Abbott Laboratories) in 0.2 ml (approximately 70 mg/kg). Anesthesia was administered to reduce risk of auto inoculation during injection. When a surgical plane of anesthesia was reached, mice were injected i.p. with 0.5 ml of resuspended HIV-III_B culture (approximately 5×10^8 MT-4 cells), using forceps for manipulations of the mouse and needle. Mice were returned to sterilized cages and allowed to recover.

AZT was suspended in Hanks' balanced salt solution (HBSS) and filter sterilized. Medications were delivered i.p. in volumes of 0.5 ml. Mock-treated mice received 0.5 ml of HBSS without AZT. The initial medication was administered 6 h postinoculation, and was continued once daily for 7 total doses.

At 7-day intervals following inoculation, mice were sacrificed by cervical dislocation and the spleens were removed, weighed and placed in 24-well tissue culture plates on ice. Cold lysis buffer (300 mM NaCl, 50 mM Tris-HCl pH 7.6, 0.5% Triton X-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1.8 mg/ml iodoacetamide) was added in 1.0-ml volumes to each spleen. Using forceps, the spleens were minced and homogenized in lysis buffer and then incubated for 2 h at 4°C. A volume of 200 μl of liquid was removed from each well and assayed for p24 antigen content using a commercial kit (Abbott Laboratories, North Chicago, IL). Each assay was standardized using p24 antigen standards (Abbott Laboratories) of known concentration. Based on p24 concentrations in the spleen homogenates, p24 concentrations were calculated both per gram of spleen tissue, and per whole spleen. (Tables 1–3). The detection limit of the assay was approximately 5 pg of p24/g of spleen. The day-to-day variance of the p24 antigen assay was less than 2% at 12.5 pg/ml and less than 8% at 100 pg/ml.

Live HIV was recovered from spleen or peritoneal wash by using a co-cultivation technique. Spleens or peritoneal washes were collected in RPMI 1640 media plus 10% fetal bovine serum. Spleens were homogenized with sterile forceps as described before. The peritoneal or spleen cell suspensions were washed in media once and then added to MT-4 monolayer cultures in 24 well plates (Falcon). The plates were incubated at 37°C for 4 days and then tested for p24 antigen in the culture supernatant. Cells were passaged and fresh MT-4 cells added as described previously (Gallo et al., 1984). Additional p24 assays were performed on cell supernatants from cultures for up to 14 days after the initial mouse cell harvest.

2.3. Pharmacokinetic evaluations

Utilizing a protocol identical to the therapeutic trials, CF1 mice received a 5 or 50 mg/kg i.p. dose of AZT once daily for 3 consecutive days. Spleen and serum samples were collected from groups of 5 mice 0.25, 0.5, 1, 1.5, 2, 3 and 6 h after the third dose. Spleens were weighed and homogenized in 1 ml of PBS. Spleen homogenates (0.2 ml) were mixed with 4 vol. of methanol to precipitate the proteins. Following centrifugation, the supernatants were lyophilized to dryness. The spleen samples were reconstituted with 0.2 ml water for HPLC analysis. The same methods were used to prepare spleen standards from untreated mice. The spleen standards were spiked with AZT prior to

reconstitution with water for HPLC analysis. The serum samples (0.2 ml) were mixed with 0.05 ml of 2 M perchloric acid to precipitate the proteins. Following centrifugation, the supernatant from the serum precipitate was analyzed by HPLC.

AZT was separated from the serum and spleen homogenate contaminates using reverse-phase HPLC on a 5 cm × 4.6 mm 3-μm ODS2 column with an acetonitrile : 0.02 M acetate buffer (pH 5.56) (5 : 95 v/v) mobile phase at a flow rate of 1.0 ml/min with UV detection of the 100-μl injection at 267 nm. The method was linear (correlation coefficient > 0.99) over the range of 0–20 μg/ml with a mean percent standard deviation of $< 3\%$ for the analysis of triplicate standards at 6 different concentrations. The estimated detection limit was 0.05 μg/ml for serum and 0.1 μg/g for spleen samples. The AZT concentration of each sample was calculated by least squares linear regression (unweighted) of the peak area of spiked mouse serum or spleen standards versus concentration. The mean parent AZT concentration data were submitted to multi-exponential curve fitting using the program CSTRIP (Sedman and Wagner, 1976) to provide initial parameter estimates for NONLIN84 (Statistical Consultants, 1986). Model selection was based on comparisons of data fitted to bi-exponential and tri-exponential equations. The initial weighting scheme was concentration^{-1}. Area under the curve (AUC) values were calculated by the trapezoidal method over the time course of the study. Total hemoglobin in the spleen tissue homogenate was determined using Drabkin's Reagent (Nelson and Morris, 1984). Spleen homogenate concentrations were adjusted for drug concentrations present in residual blood.

2.4. Biologic safety

All manipulations and inoculations with HIV virus were performed in a class 2 biologic safety cabinet in a BSL-3 facility. Customary BSL-3 precautions were followed during all procedures with live or potentially live virus. Whenever possible, forceps were used for manipulations involving syringes containing HIV cultures. Serologic surveillance was conducted on all workers during the course of the investigation.

3. Results

3.1. Time course trials

HIV infection in reconstituted SCID mice was stable for 4 weeks postinoculation, based on the percentage of mice with detectable p24 antigen and on the p24 antigen burden in the splenic tissue (Table 1). The number of mice with detectable viral burden ranged from 89% (8/9) on day 14 to 70% (7/10) on day 28 postinoculation. The quantity of HIV p24 antigen in SCID spleen tissue ranged from 911 pg p24/g spleen on day 14 to 499 pg p24/g spleen on day 21. Seven to 10 mice were assayed at each time point.

Mice injected with a killed inoculum of HIV equivalent in quantity to live HIV inoculums did not yield detectable p24 antigen in spleen tissue beyond 24 h postinoculation (data not shown).

Table 1
Mean HIV p24 antigen burden in spleens of SCID mice 7–28 days postinoculation

Days post-inoculation	Infected/total	pg p24 per whole spleen [a]		pg p24/g spleen [a]	
		Mean ± S.E.M.	Range	Mean ± S.E.M.	Range
7	8/10	26.6 ± 18.3	(2.6–52.0)	710 ± 614	(47–1531)
14	8/9	35.3 ± 42.0	(3.8–98.6)	911 ± 1211	(24–2738)
21	6/7	19.5 ± 15.0	(2.6–37.8)	499 ± 403	(60–1144)
28	7/10	38.4 ± 33.9	(8.0–92.0)	622 ± 426	(181–1227)

[a] Excluding non-infected animals from calculation.

P24 antigen was detected infrequently and in low concentrations in mouse plasma. Peritoneal wash was infrequently positive for p24 antigen when assayed beyond 24 h postinoculation (data not shown). Live HIV was recovered from suspensions of spleen cells from SCID mice. Spleen cells suspended in saline cultured with MT-4 cells resulted in detectable p24 antigen in the culture supernatants. Over 14 days of incubation with the addition of fresh MT-4 cells to the co-cultivation system were needed to obtain detectable p24 levels (data not shown). The murine spleen cells and debris appeared to initially inhibit the MT-4 cell cultivation system. This technique was too cumbersome to allow quantitative evaluations of HIV burden (data not shown).

3.2. AZT efficacy trials

Daily i.p. AZT therapy was associated with a decrease in the quantity of HIV in the spleens of mice assayed 7 days postinoculation (Table 2). AZT therapy administered at 0.5, 5.0, or 50.0 mg/kg/day for 7 days produced 18, 52, and 95% reductions in p24 antigen burden per gram of spleen, respectively. Only the 50 mg/kg dosage was associated with a greater than one log reduction in p24 burden. A similar percentage of AZT-treated and HBSS mock-treated mice yielded detectable p24 burdens.

In a subsequent trial, AZT therapy at 50 mg/kg/day for 7 days produced a 92% reduction in the quantity of p24 per gram of spleen tissue (Table 3). The percent of mice with detectable p24 was similar in AZT-treated (13/19) and HBSS-treated (12/15) mice. The mean quantity of p24 antigen was 1566 pg/g spleen in the HBSS treated control mice, the highest burden observed in this investigation. The range of individual

Table 2
Effect of AZT therapy on mean HIV p24 antigen burden in spleens of SCID mice 7 days postinoculation

Group	Infected/total	pg p24/whole spleen [a]		pg p24/g spleen [a]	
		Mean ± S.E.M.	Range	Mean ± S.E.M.	Range
HBSS	9/10	51.0 ± 28	(1.1–252.3)	413 ± 247	(31–4204)
AZT 0.5 mg/kg	8/10	35.2 ± 31	(1.6–252.3)	336 ± 320	(7–5148)
AZT 5.0 mg/kg	7/8	28.3 ± 19	(2.9–137.8)	199 ± 122	(15– 660)
AZT 50.0 mg/kg	8/9	4.8 ± 1	(2.9– 12.1)	18 ± 10	(12– 170)

Note: HBSS, Hank's balanced salt solution.
[a] Excluding non-infected animals from calculation.

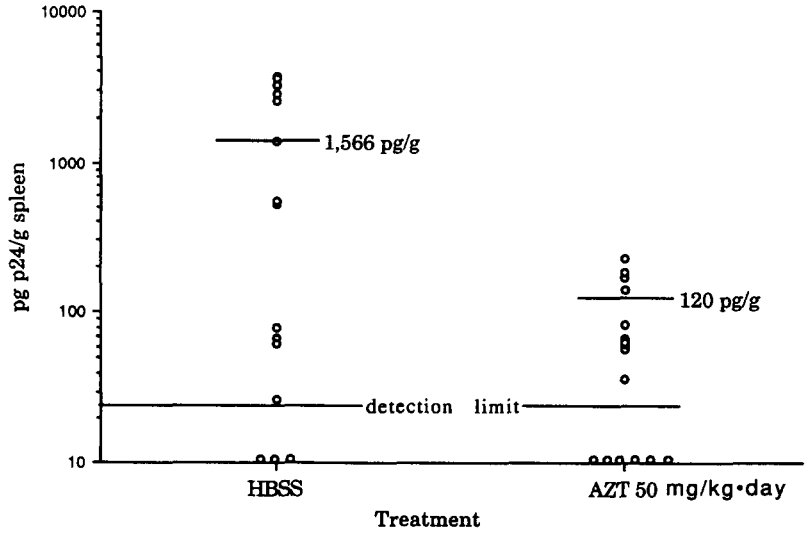

Fig. 1. Concentrations of p24 antigen in spleens of mice infected with HIV and treated with AZT.

p24 concentrations per gram of spleen varied by approximately two logs in mock-treated mice (Fig. 1).

In approximately one-quarter of the trials, there was a general failure to initiate HIV infection and few or none (< 25%) of the mice yielded detectable virus (data not shown). When HIV infection was successful, over 75% of control (mock-treated) SCID

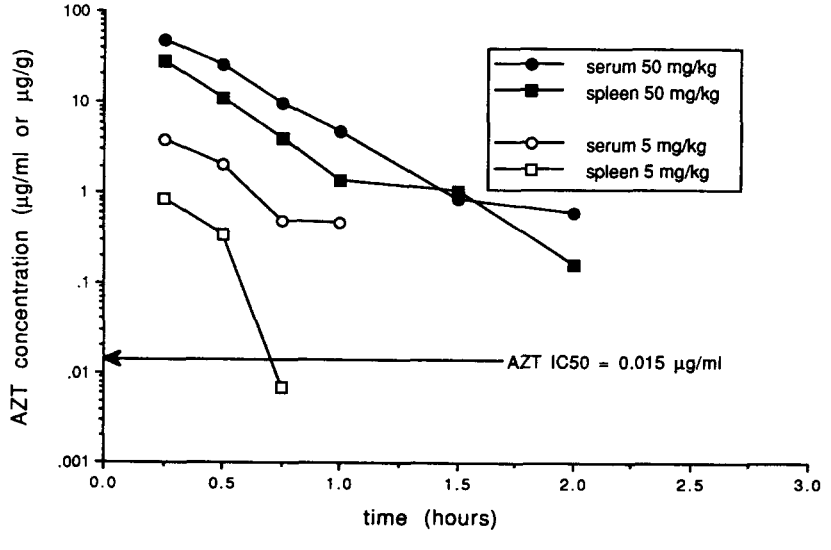

Fig. 2. Serum and splenic concentrations of AZT in mice following a 50 or 5 mg/kg intraperitoneal dose.

mice yielded detectable virus. For this reason, only trials in which 80% of the control mice yielded detectable p24 antigen were used for evaluation of AZT therapy. The concentration of p24 in spleens of control mice varied by approximately 4-fold between trials.

3.3. Pharmacokinetic evaluation of AZT in mice

Following a 50 mg/kg i.p. dose, peak serum and spleen concentrations of AZT were 47 and 27 μg/ml or g, respectively, which is approximately 1000-fold higher than the in vitro IC_{50} values (Table 4). Serum and splenic concentrations of AZT more than 10-fold greater than the IC_{50} concentrations were maintained for at least 2 h postinjection (Fig. 2). The limit of HPLC detection of AZT (approximately 0.1 μg/ml in spleen homogenate) was significantly higher than the in vitro IC_{50} value of AZT (0.015 μg/ml). However, projection of serum and spleen concentrations indicate that the concentrations of AZT likely decreased below the IC_{50} value within 3 h following a 50 mg/kg dose. Mice dosed at 5 mg/kg also produced serum and spleen concentrations well in excess of the in vitro IC_{50}. The length of time that AZT concentrations were maintained above the IC_{50} value was decreased by approximately two-fold in mice dosed at 5 mg/kg compared to mice dosed at 50 mg/kg. The plasma and spleen time profiles were fit to a two-compartment open model.

4. Discussion

HIV was present in sufficient concentration in splenic tissue of SCID mice to be assessed by standard clinical p24 antigen detection techniques. This model utilized a large inoculum of HIV and multiple reconstitutions with human lymphocytes, both before and after inoculation. These modifications may have produced the high yield of HIV detectable by p24 assay. The infection was detectable for at least 4 weeks, allowing the evaluation of AZT therapy. A 1-week course of AZT therapy was used, which allowed for a rapid evaluation of drug efficacy. Mice inoculated with killed virus did not produce detectable p24, which suggests that viable virus was needed to maintain detectable p24 concentrations. This model may present an improvement for the preclinical testing of HIV therapeutics.

Table 3
Effect of AZT therapy on mean HIV p24 antigen burden in spleens of SCID mice 7 days postinoculation

Group	Infected/total	pg p24/whole spleen [a]		pg p24/g spleen [a]	
		Mean ± S.E.M.	Range	Mean ± S.E.M.	Range
HBSS	12/15	50.5 ± 45	(3.5–149.3)	1566 ± 1532	(27–3728)
AZT 50 mg/kg	13/19	8.5 ± 7	(1.4– 23.7)	120 ± 88	(37– 232)

Note: HBSS, Hank's balanced salt solution.
[a] Excluding non-infected animals from calculation.

AZT therapy produced a decrease in the quantity of detectable p24 antigen present 7 days after inoculation. While a dose response was noted, only the 50 mg/kg/day dose of AZT was associated with a one log reduction in p24 antigen burden. In two trials, AZT therapy at 50 mg/kg/day produced similar decreases (95 and 92%) in p24 concentration (Tables 2 and 3). Lower doses of AZT (5 or 0.5 mg/kg/day) produced less than a one log reduction (52 and 18%, respectively) in p24 concentration. The p24 concentration in control mice (HSBB-treated) was approximately 4-fold higher compared to the previous trial (Table 3). These results are similar to clinical results of AZT therapy. Significant decreases in p24 serum concentration from 111 to 46 pg/ml (Chaisson et al., 1988) and reversions of serum p24 concentration below detection levels (Wolf et al., 1988) are initially associated with clinical AZT therapy.

McCune et al. (1990b) demonstrated that several weeks of AZT therapy initiated prior to infection reduced the proportion of mice with HIV burden as detected by PCR techniques. In the trials reported here, a shorter course of AZT therapy initiated after infection was effective. The reason for these different profiles of drug efficacy may be due to larger inoculum and viral burden in this model. Mosier et al. (1991) inoculated with approximately 5×10^6 (infected) cells per mouse, Namikawa et al. (1988) inoculated with 400–4000 infectious units in a direct intrathymic injection, while Wetherall (1990) infected with 2×10^5 tissue culture infectious doses. The quantity of virus used by these investigators was probably less than the 5×10^8 cells per mouse inoculum used in this investigation. A 1-week course of AZT treatment initiated after infection was sufficient to achieve detectable reductions in HIV p24 antigen burden in the modified SCID mouse model.

The pharmacokinetic data for AZT in mice is similar to that reported previously by other groups. The plasma $t_{\frac{1}{2}}$ value following a 50 mg/kg i.p. dose was 0.95 h (Table 4). Doshi et al. (1989) reported a $t_{\frac{1}{2}}$ value of 1.19 h after a 50 mg/kg i.v. dose, while Trang et al. (1993) reported plasma $t_{\frac{1}{2}}$ values of approximately 0.33 h following i.v. doses of 15, 30, or 60 mg/kg. In man, AZT is routinely dosed 5 times daily, producing sustained serum concentrations of 10 to $1000 \times IC_{50}$ (Klecker et al., 1987; Parks et al., 1988). In mice, an i.p. dose of 50 mg/kg produced serum and spleen concentrations of 5000 to $100 \times IC_{50}$ during the period 0–2 h postadministration; however AZT was not detected beyond 2 h (Fig. 2). Pharmacokinetic profiles of effective and ineffective

Table 4
Pharmacokinetic evaluation of AZT in mice following a 50 or 5 mg/kg i.p. dose

Sample	$t_{\frac{1}{2}}$ (h)	C_{max} (μg/ml or g)	T_{max} (h)	$AUC_{0-\infty}$ (μg/h/ml or g)
50 mg/kg				
Plasma	0.95	47.10	0.25	23.18
Spleen	0.67	27.50	0.25	11.85
5.0 mg/kg				
Plasma	n.d.	3.75	0.25	1.758
Spleen	n.d.	0.86	0.25	0.297

Note: n.d., not determined.

dosages for experimental therapeutics would be a valuable tool for planning clinical evaluations.

A quantitative evaluation of HIV drugs in vivo would be useful for determining therapeutic potential. Correlation of in vitro, pharmacokinetic, and efficacy data is important for preclinical evaluations. Common parameters used for anti-HIV compounds include C_{max}/IC_{50}, AUC/IC_{50}, and time with drug concentrations in plasma maintained above MIC values ($t > IC_{50}$). These parameters can be compared to drug efficacy in dose-ranging trials to determine key factors.

There was a clear dose response to AZT therapy at 0.5, 5, and 50 mg/kg/day. A plot of log dose versus percent reduction in p24 antigen burden yielded a linear plot ($r = 0.994$, data not shown). Of interest is the 10-fold reduction in p24 antigen burden from 199 pg/g spleen to 18 pg/g spleen as the daily dosage increased from 5 to 50 mg/kg/day. This increase in dosage produced a 30 × increase in the C_{max}, a 40 × increase in the AUC value and a 4 × increase in the $t > IC_{50}$. Additional dose–response trials are needed to draw correlations between pharmacokinetic, in vitro, and in vivo efficacy of AZT in this SCID mouse model.

The detection of p24 antigen at a concentration in excess of 600 pg/g of spleen 4 weeks postinoculation (Table 1) suggests that active HIV replication in the mice was a factor in maintaining high p24 concentrations. The lack of detectable p24 following injection of killed virus further suggests that p24 antigen concentrations were maintained through HIV replication. Viable HIV virus was recovered from the spleens of infected SCID mice, but the technique was too cumbersome to be routinely used in a screening program. Active HIV replication presents a sensitive target for drug therapy and a model for drug development (Spertzel, 1989). The efficacy of a 7-day course of AZT therapy was likely aided by active HIV replication in the reconstituted SCID mice. Similar periods of antiviral therapy are associated with efficacy in viral infections of mice including HSV-1, HSV-2, CMV, and LP-BM5 (Kern et al., 1978; Boyd et al., 1988; Basham et al., 1990; Machida et al., 1990; Machida et al., 1992; Freitas et al., 1993). Active viral replication occurs in all of these models. The primary requirement for the HIV/SCID mouse model was to establish a period of HIV replication, allowing for a rapid response to AZT therapy.

This model, as do others for this disease, presents difficulties for the evaluation of potential HIV therapeutics. Unfortunately, detectable p24 concentrations were rarely achieved in plasma, necessitating a terminal assay for assessment of drug efficacy. Approximately one-quarter of the trials did not yield detectable splenic p24 in an acceptable proportion of mice. These unsuccessful trials were apparent when less than 25% of the spleens from mock-treated control mice yielded detectable p24 antigen. Evaluation of drug efficacy was not possible in these trials. Since the status of HIV infection is not apparent until trial conclusion, experimental therapeutics, often available in small quantities, could be consumed without the generation of useful data. Within each acceptable trial, the infection rate for mock-treated mice ranged from 70 to 100%. Under these conditions, larger numbers of mice per group and replicate trials are needed for adequate evaluation of compounds. The mean concentration of p24 per gram of spleen varied in untreated mice by 4-fold between trials, complicating comparisons between different trials.

In addition, biohazard issues are of considerable concern since this model requires the injection of live HIV virus into mice. Additionally, sharp instruments are used in the dissection of HIV-infected mice. The p24 burdens and the recovery of live virus in the spleens of the mice suggest that this material was infectious. Conservatively, strict BSL-3 containment conditions should be maintained for both in vitro and in vivo procedures, although the biohazard of HIV-infected SCID mice is considered no greater than that of an AIDS patient (Milman and D'Souza, 1990).

The differences in viral p24 antigen burden and rate of HIV infection between trials is most likely due to the inherent variables of the model. Since HIV is not able to directly colonize murine species, a human graft must be implanted. In this model, the graft is dependent upon successful colonization of murine spleen by human CD4 + lymphocytes. The success rate of xenografts varies considerably, even in genetically immunodeficient mice (Fodstad, 1988; Igarashi et al., 1989). Secondly, the HIV inoculum must localize and replicate in the engrafted human cells, which are a minority fraction of the cells in the murine spleen (Mosier et al., 1988). Finally, the antiviral effect of drug therapy must be distinguished from the potential antigraft effect, which could cause an apparent decrease in HIV viral burden in the mice. The variation in viral burden and infection rate suggests that this model is not insensitive to factors independent from antiviral therapy.

The value of this mouse model of HIV infection is that more rapid evaluation of potential HIV therapeutics is possible. These preclinical trials in SCID mice are considerably less costly and time consuming than are trials in humans. Factors difficult to model in vitro, such as serum binding, tissue penetration, and pharmacokinetics, have significant impact on drug efficacy in animal experiments, allowing better evaluation of anti-HIV compounds prior to clinical trials. The rapid evaluation of experimental agents or drug combinations prior to commitment of resources for clinical studies could reduce the cost and time required for research and development of HIV therapeutics.

Acknowledgements

The work of Ken Jarvis, Mike Mitten, Lenette Paige, Tom Hutch, and Dr. Nate Shipkowitz, (Microbiology), Dr. Kennan Marsh (Drug Analysis), Sudthida Vasavanonda and Dr. Terry Robins (Virology), and Todd Howard, Debbie Henning, and John Freeman, (Animal Care) is gratefully acknowledged.

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