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Antiviral Research 27 (1995) 151–163

Antiviral Research

A simple and rapid method for preliminary evaluation of in vivo efficacy of anti-HIV compounds in mice

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Received 10 October 1994; accepted 3 January 1995

Abstract

In vivo efficacy of anti-HIV compounds is affected by various factors such as bioavailability, metabolism, clearance, and toxicity. Here we report a simple and rapid method that might be useful for preliminary evaluation of in vivo efficacy of anti-HIV compounds. MT-4 cells carrying proviral HTLV-1 were infected with HIV-1 in vitro and injected into the peritoneal cavity of SCID mice or BALB/c mice. Inoculated cells survive for 1–2 days, and support one to two cycles of viral replication which can be monitored by RT activity or p24 content in the supernatants of peritoneal wash fluids. Test compounds were administered either orally or subcutaneously. AZT, DDC and DDI, the nucleoside-type RT inhibitors currently in clinical use, all showed potent anti-HIV-1 activities in this mouse/MT-4 assay. HEPT (E-EBUdM), a non-nucleoside RT inhibitor, also showed potent anti-HIV-1 activity in vivo, whereas TIBO (R 82913), another non-nucleoside RT inhibitor, was less active. In protease inhibitors KNI-272 and Ro 31-8959 showed good in vivo activities, while KNI-144, a compound closely related to KNI-272, showed poor in vivo activity. This mouse/MT-4 assay, although having a number of shortcomings as an animal model for HIV-1 infection, may be of some practical utility for preliminary evaluation of in vivo efficacy of potential anti-HIV compounds.

Keywords: HIV-1;Antiviral compound;mouse

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SSDI 0166-3542(95)00004-6

1. Introduction

The pandemic of AIDS requires rapid development of effective anti-HIV drugs. Candidate compounds are first selected by in vitro assays, but the efficacy of such compounds in vivo is greatly affected by factors such as bioavailability, metabolism, clearance, and toxicity that cannot be foreseen by in vitro assays. Few animal models are currently available for evaluation of anti-HIV compounds in vivo. Some may employ the combination of animal retroviruses and natural hosts (Ruprecht et al., 1986; Daniel et al., 1987; Pedersen et al., 1987). These models are, however, only applicable to compounds that are commonly effective against animal retroviruses and HIV. Recently, severe combined immune deficiency (SCID) mice transplanted with human lymphoid cells were introduced as a mouse model allowing HIV infection (McCune et al., 1990; Torbett et al., 1991). It may be, however, difficult to maintain a constant supply of such mice for routine evaluation of a number of candidate compounds. Here we describe a simple and rapid mouse method that might be potentially useful for the preliminary evaluation of candidate anti-HIV compounds for in vivo efficacy.

2. Materials and methods

2.1. Compounds

AZT (3′-azido-2′,3′-dideoxythymidine) (Fischl et al., 1987) was purchased from Sigma (St. Louis, Mo.). DDC (2′,3′-dideoxycytidine) (Mitsuya et al., 1985) and DDI (2′,3′-dideoxyinosine) (Yarchoan et al., 1989) were purchased from Calbiochem (San Diego, CA). TIBO (R 82913) ((+)-*S*-4,5,6,7,-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-thione) (Pauwels et al., 1990) was purchased from PharmaTech International Inc. (West Orange, NJ). HEPT (E-EBUdM) (6-(3,5-dimethyl benzyl)-1-ethoxymethyl-5-ethyluracil) (Baba et al., 1991), and the protease inhibitors Kynostatin (KNI)-272 and KNI-144 (Mimoto et al., 1992), and Ro 31-8959 (Roberts et al., 1990), were synthesized in our laboratories. Table 1 summarizes the compounds used in the present study and their inhibitory activities on HIV-1 replication determined by a standard in vitro MTT assay (see below).

2.2. Cells and virus

MT-4 (Harada et al., 1985) and M8166 (Shibata et al., 1991) are human T-cell lines, established by HTLV-1-induced transformation. They are highly sensitive to HIV-1 infection, resulting in rapid and strong cytopathic effects. The stock of HIV-1 was produced as follows. Molt-4 cells persistently infected with HIV-1(IIIB strain) were cultured for 3 days and culture supernatants were collected. After removal of cells by centrifugation at 900 *g* for 5 min, the supernatants were passed through membrane filters (0.45 μm pore size) and stored at −80°C until use. The infectivity of the stock virus which was measured by a plaque assay as described previously (Harada et al., 1985) was 1.2×10^5 plaque forming units/ml.

Table 1
Summary of in vitro anti-HIV-1 activities of compounds used in the present study

Compound	MTT assay [a]		
	CC_{50} [b]	EC_{50} [c]	S.I. [d]
AZT	24	0.0007	34,000
DDC	1.6	0.0034	470
DDI	> 100	0.46	> 220
TIBO (R82913)	29	0.056	520
HEPT (E-EBUdM)	> 100	0.00046	> 220,000
KNI-272	29	0.015	1,900
KNI-144	31	0.09	340
Ro 31-8959	6.8	0.0083	820

[a] Data are mean values of at least two determinations.
[b] 50% Cytotoxic concentration (μg/ml).
[c] 50% Effective concentration (μg/ml).
[d] Selectivity index (S.I.) or the ratio of CC_{50} and EC_{50}.

2.3. *In vitro assay for anti-HIV-1 compounds*

Each compound was evaluated for anti-HIV-1 activity in vitro by inhibition of the virus-induced cell death essentially as described previously (Pauwels et al., 1988) with slight modifications. Briefly, MT-4 cells were suspended in culture medium at 3.5×10^5 cells/ml. The cell suspension (100 μl) was added to each well of 96-well flat-bottom microtiter plates containing test compounds (50 μl/well). HIV-1(IIIB) was added to each well at a multiplicity of infection (m.o.i.) of 0.0008 (50 μl/well). After a 5-day incubation at 37°C, the viability of MT-4 cells was determined by the MTT method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

2.4. *HIV-1 growth in vivo*

CB-17 scid/scid (SCID) mice, BALB/c nude mice and BALB/c mice, all female, were purchased from Nihon Clea (Tokyo, Japan). The animals were housed in microisolator cages at 25 ± 1°C and freely supplied with food and water. MT-4 cells were infected with HIV-1(IIIB) at a m.o.i. of 0.002. After infection for 2 h at 37°C, the cells were washed 3 times with phosphate-buffered saline (PBS) at 4°C to remove free virions. The cells were suspended in PBS and inoculated into the peritoneal cavity of each mouse (5×10^7 cells in 0.3 ml/mouse). At the indicated time points, 5 ml of PBS was injected into the peritoneal cavity of each mouse and peritoneal wash fluids were collected. After removal of cells by centrifugation, the supernatants were stored at −20°C until the reverse transcriptase (RT) assay and ELISA for p24 antigen (see below). The survival of MT-4 cells in the peritoneal cavity was examined by the trypan blue dye exclusion test.

2.5. *RT assay and ELISA for p24*

RT assay was carried out as described previously (Wu et al., 1991) with slight modifications. Briefly, a 100-μl reaction mixture contained 50 mM Tris-HCl, pH 8.3, 150 mM KCl, 10 mM $MgCl_2$, 0.1% Nonidet P-40, 10 mM dithiothreitol, 5 mg/ml

poly(rA), 5 mg/ml $(dT)_{12-18}$, 1 mCi [^{3}H]dTTP and 10 μl of each sample. After incubation at 37°C for 3 h, the reaction mixtures were chilled on ice and passed through a DEAE-Filtermat (LKB-Pharmacia, Turku, Finland) using a cell harvester. After washing with 5% Na_2HPO_4 and H_2O, radioactivity on the filters was determined by LKB Beta Plate scintillation spectroscopy. The amount of p24 antigen was assayed by using a sandwich-type ELISA kit (Abbott Laboratories, Chicago, IL).

2.6. Treatment of mice with anti-HIV compounds

For oral administration, each compound was dissolved or suspended in 3% Arabic gum–sterile water and administered directly into the stomach by using a mouse stomach cannula (0.2 ml/mouse). For subcutaneous administration, AZT, DDC, and DDI were dissolved in PBS, whereas TIBO, HEPT, KNI-272, KNI-144, and Ro 31-8959 were dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mg/ml and appropriately diluted with fetal calf serum (FCS). Inhibition rate of HIV-1 replication was calculated as follows:

$$\text{Inhibition}\ (\%) = (1 - P/A) \times 100$$

where P is the level of specific RT activity in the presence of drug and A is the level of specific RT activity in the absence of drug.

2.7. Pharmacokinetic study

Drugs were administered orally to ICR or BALB/c mice at a dose of 50–250 mg/kg body weight. Blood samples were taken at 30, 120, and 240 min following drug administration by heart puncture under anesthesia with diethyl ether. The sera were separated and stored at −20°C until analysis. Compounds in 100 μl of the serum samples were extracted with 1 ml of ethylacetic acid by shaking for 10 min. After centrifugation for 5 min, the organic layers were evaporated. The residues were dissolved with 25 μl of DMSO and diluted with 125 μl of 0.1% trifluoroacetic acid (TFA) in deionized water. One hundred ml of each sample was injected into an HPLC system composed of a pump model 126 (Beckman, CA), a UV-detector model 166 (Beckman, CA), a WISP autosampler model 712 (Waters, MA) and a column of TSK gel ODS-80Ts (Tosoh, Tokyo, Japan) (4.6 i.d. × 150 mm). The flow rate was 0.8 ml/min. The gradient changed linearly from 20 to 80% acetonitorile in water containing 0.1% TFA for 30 min for TIBO and KNI-272, and from 0 to 30% acetonitorile in water containing 0.1% TFA for 30 min for AZT. HEPT was determined with isocratic elution of 40% acetonitorile in water containing 0.1% TFA. The compounds were detected by UV-spectrometry: 267 nm for AZT, 230 nm for KNI-272, 265 nm for HEPT, and 310 nm for TIBO.

3. Results

3.1. Replication of HIV-1 in MT-4 cells transplanted in the peritoneal cavity of mice

In the SCID-hu model, mice are first reconstituted with human lymphoid tissues and then infected with HIV-1 (McCune et al., 1990). The efficacy of an anti-HIV-1

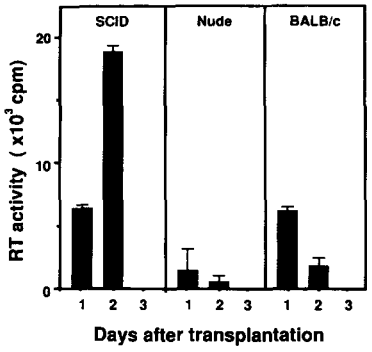

Fig. 1. HIV-1 replication in MT-4 cells intraperitoneally transplanted in mice. SCID mice, BALB/c nude mice, and normal BALB/c mice, all female and 12 weeks old, were used. MT-4 cells were infected with HIV-1(IIIB) and transplanted into the peritoneal cavity of mice. RT activity in peritoneal wash fluids was monitored on days 1, 2 and 3 after transplantation. All values are given as the mean ± S.D. of 3 mice.

compound may thus be assessed in a situation mimicking natural infection in humans. The model may, however, be difficult for routine use. We reasoned that the factors affecting in vivo efficacy of an anti-HIV-1 compound such as bioavailability and effective half-life might be tested in mice just transplanted with HIV-1-infected human T-cell lines. We chose MT-4 cells carrying proviral HTLV-1, because MT-4 cells support rapid growth of HIV-1 (Harada et al., 1985). HIV-1 replication in the peritoneal cavity was examined in SCID mice, BALB/c mice, and BALB/c nude mice, all at the

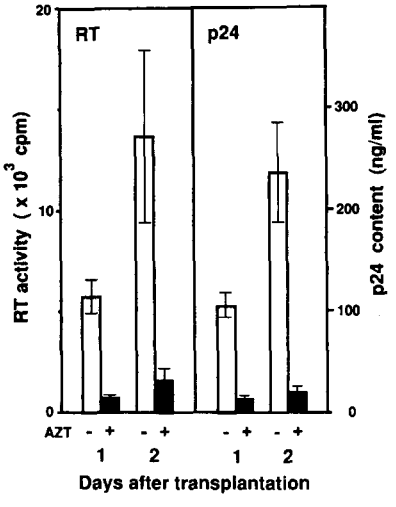

Fig. 2. Inhibitory effect of AZT on HIV-1 replication in MT-4 cells transplanted in SCID mice. AZT was administered orally at a dose of 50 mg/kg/day to the mice. RT activity and p24 antigen in peritoneal wash fluids were monitored on days 1 and 2 after transplantation. Open bar, control; closed bar, AZT-treated. All values are given as the mean ± S.D. of 3 mice.

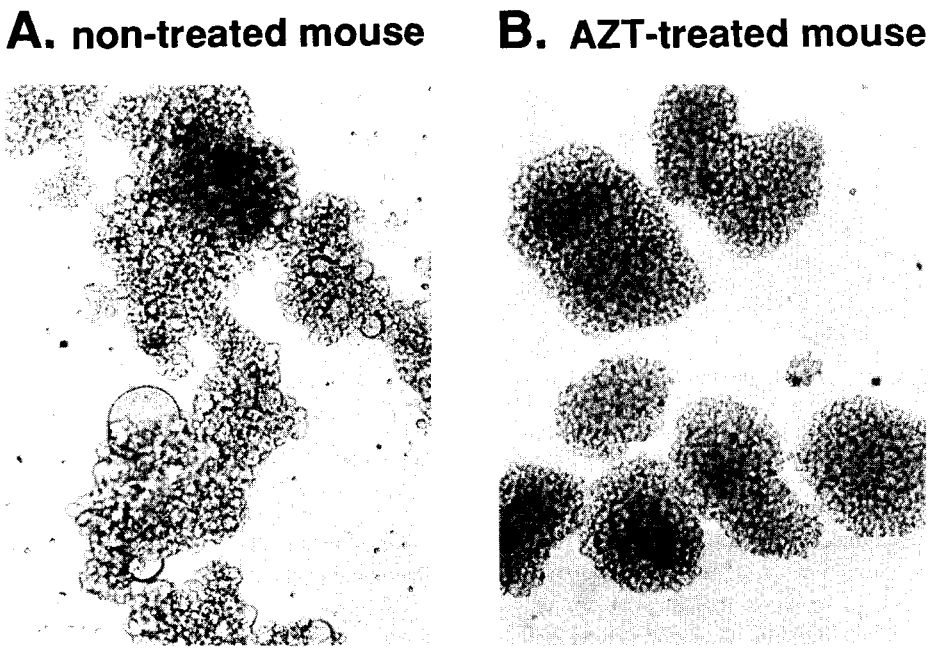

Fig. 3. Detection of HIV-1 in sera of mice injected with HIV-1-infected MT-4 cells. M8166 cells (5×10^4) were cultured with addition of sera (25 μl) from (A) non-treated mice or (B) AZT-treated mice (50 mg/kg), and photographed 7 days later under phase-contrast microscopy ($\times 90$).

age of 12 weeks, after intraperitoneal inoculation of HIV-1-infected MT-4 cells. As shown in Fig. 1, the highest levels of HIV-1 replication as monitored by RT activity in the supernatants of peritoneal wash fluids were obtained in SCID mice. Following the transplantation of HIV-infected MT-4 cells, the levels of RT activity increased until day 2, but markedly declined by day 3. Using BALB/c mice, RT activities on day 1 were more or less similar to those of SCID mice, but already declined by day 2. Much less efficient growth was observed in the nude mice. HIV-1 p24 and infectious particles were also detected in sera of mice transplanted with HIV-1-infected MT-4 cells, indicating the occurrence of viremia (data not shown) (see also Figs. 2 and 3).

To understand the rapid decline of RT activity after 2–3 days post-transplantation, the survival of uninfected MT-4 cells in the peritoneal cavity was examined next (Table 2). In the case of SCID mice, the recovery of viable cells increased until day 2, but rapidly decreased by day 3. The recovery of cells from BALB/c mice already decreased by day 2. The cell recovery from the nude mice was the lowest, probably because of strong NK activity in this strain. These values were roughly parallel to the levels of HIV-1 replication in these mouse strains (see Fig. 1), suggesting that the survival of MT-4 cells in the peritoneal cavity was the primary determinant for the level of HIV-1 replication. In most of the following experiments, we used SCID mice or BALB/c mice at the age of more than 10 weeks, and determined the level of HIV-1 replication 24 h after transplantation.

Table 2
Recovery of MT-4 cells from the peritoneal cavity of transplanted mice

Mouse strain [a]	Number of viable cells recovered ($\times 10^7$ cells) [b]		
	Day 1	Day 2	Day 3
SCID	1.4±0.1	1.8±0.2	0.4±0.06
Nude	0.9±0.3	0.6±0.3	0.3±0.06
BALB/c	1.3±0.1	0.7±0.02	0.3±0.04

[a] Each mouse was inoculated i.p. with 5×10^7 MT-4 cells.
[b] Three animals were used for each determination and values were given as mean ± S.D.

3.2. Inhibitory effect of AZT on HIV-1 replication in MT-4 cells in mice

To see whether HIV-1 replication in MT-4 cells in the mouse peritoneal cavity could be used for an in vivo evaluation of anti-HIV compounds, the effect of AZT, a potent inhibitor of HIV-1 RT (see Table 1), was tested. AZT was administered by the oral route to SCID mice (50 mg/kg) immediately after transplantation of the HIV-1-infected MT-4 cells. HIV-1 replication was monitored by the RT assay as well as p24 ELISA. As shown in Fig. 2, RT activities recovered in the peritoneal wash fluids were reduced dramatically by AZT treatment (89% inhibition by day 1 and 91% inhibition by day 2). The inhibitory effects of AZT were not due to direct cytotoxicity of AZT, since the recoveries of viable MT-4 cells from AZT-treated mice were not less than those from non-treated mice (data not shown). Since residual AZT in the peritoneal wash fluids might have contributed to RT inhibition in the in vitro RT assay, we further determined the content of viral p24 in the peritoneal cavity. The amounts of p24 produced in the peritoneal wash fluids were also dramatically reduced by AZT and the levels of reduction were quite comparable to those of RT inhibition (90% inhibition by day 1 and 92% inhibition by day 2). To monitor viremia, blood samples were collected by heart puncture and centrifuged to obtain sera. M8166 cells were inoculated with these sera. Syncytia developed about 1 week later in cells inoculated with serum samples from untreated mice (Fig. 3A). RT activity was detected in the culture supernatants and

Table 3
Serum concentrations of various anti-HIV drugs after oral administration to mice

Time (h) [a]	Serum concentration (μg/ml) (mean ± S.D.)			
	AZT [b]	KNI-272 [c]	HEPT [d]	TIBO [e]
0.5	20 ±5.4	17 ±3.1	2.0±0.6	1.6±0.8
2	2.9±2.6	2.8±3.9	1.8±0.3	2.0±0.8
4	0.5±0.5	0.1±0.1	ND [f]	0.5±0.4

[a] Time after oral administration.
[b] AZT was administered at a dose of 50 mg/kg body weight to 3 mice.
[c] KNI-272 was administered at a dose of 250 mg/kg body weight to 3 mice.
[d] HEPT was administered at a dose of 250 mg/kg body weight to 3 mice.
[e] TIBO was administered orally at a dose of 200 mg/kg body weight to 3 mice.
[f] Not done.

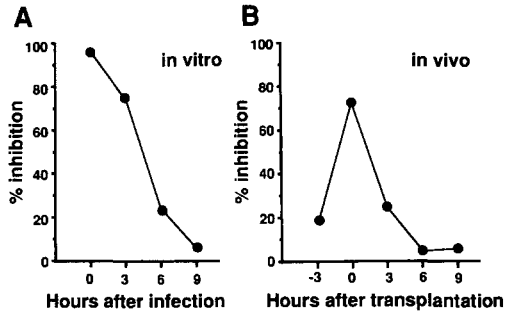

Fig. 4. Time-of-addition effect of AZT treatment on HIV-1 replication in vitro and in vivo. A: HIV-1-infected MT-4 cells were treated with AZT (1 μg/ml) starting at various time points in vitro. At 24 h after infection, RT activity in the supernatants of culture fluids was measured. Data are mean values of duplicate cultures. B: AZT (10 mg/kg) was administered orally at various time points to SCID mice transplanted with HIV-1-infected MT-4 cells. At 24 h after transplantation, RT activity in peritoneal wash fluids was determined. Data are mean values of 3 mice.

HIV-1 antigens were demonstrated in the cells by indirect immunofluorescence staining (data not shown). On the other hand, syncytium formation was delayed or negative in M8166 cells inoculated with the sera from AZT-treated mice (Fig. 3B). Furthermore, the half life of AZT in mice was found to be quite short (see Table 3). Taken together, these results indicate that the inhibitory effect of AZT was indeed due to reduction of HIV-1 replication in MT-4 cells in vivo and not due to carry-over of administered AZT.

3.3. Timing effect of AZT administration on HIV-1 replication in the mouse / MT-4 assay

Fig. 4A shows the timing effect of AZT on in vitro HIV-1 replication. It is clear that AZT is effective only in the early phase of HIV-1 replication. To test the optimal timing of AZT administration in the mouse/MT-4 assay, AZT (10 mg/kg) was given orally to SCID mice at various time points before or after transplantation of HIV-1-infected MT-4 cells and the level of replication was determined by the RT assay at 24 h after the transplantation. As shown in Fig. 4B, AZT was most effective when administered immediately after the transplantation of HIV-1-infected MT-4 cells. The inhibitory effect of AZT was dramatically reduced even when the drug was given either 3 h before or 3 h after the transplantation. We monitored the blood concentration of AZT after oral administration. As shown in Table 3, orally administered AZT reached a peak level within 30 min and rapidly declined thereafter. These results show that the reverse transcription of HIV-1 starts soon after HIV-1-infection of MT-4 and RT-inhibitors would be best administered immediately after the transplantation of HIV-1-infected MT-4 cells to obtain the highest efficacy.

3.4. Evaluation of various RT inhibitors in the mouse / MT-4 assay

Currently, AZT, DDI and DDC are being used in the therapy of patients with AIDS. These nucleoside analogues all function as chain-terminators of the RT reaction. We

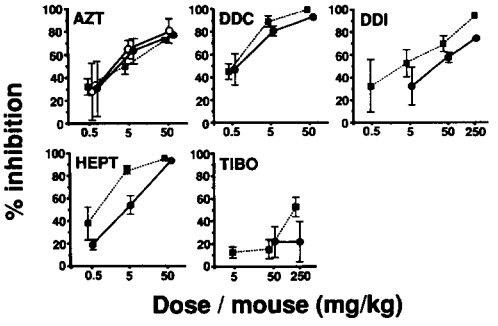

Fig. 5. Effects of various RT-inhibitors on HIV-1 replication in MT-4 cells transplanted in mice. Compounds were administered orally (●) or subcutaneously (■) to SCID mice. AZT was also administered orally to BALB/c mice (○). Drugs were administered immediately after transplantation. Data are mean values ± S.D. of 3–5 mice for two experiments.

compared the efficacy of these compounds in our mouse/MT-4 assay. Fig. 5 shows the dose–response effects of these RT inhibitors on HIV-1 replication in the mouse/MT-4 assay. Drugs were administered by the oral or subcutaneous route immediately after transplantation, and the replication of HIV-1 was monitored by RT activity measured 24 h later. No direct cytotoxicity of these drugs to MT-4 cells was observed when we compared the viability of MT-4 cells recovered from treated and non-treated mice (data not shown). Both oral and subcutaneous administration of AZT inhibited HIV-1 replication in SCID mice in a dose-dependent manner with a 50% effective dose (ED_{50}) of about 2–5 mg/kg. Oral administration of AZT also inhibited HIV-1 replication in BALB/c mice at an ED_{50} of about 2 mg/kg. DDC was also quite effective by both routes, the ED_{50} being about 0.5–0.7 mg/kg. In the case of DDI, subcutaneous administration was more effective than oral administration: the ED_{50} by subcutaneous administration was about 3 mg/kg, while the ED_{50} by oral administration was about 30 mg/kg.

Even though DDI was about 500 times less potent than AZT in the in vitro assay (see Table 1), DDI administered by subcutaneous route in the mouse/MT-4 assay was almost as effective as AZT. This may be related to the relatively long half-life of dideoxyadenosine triphosphate, the active metabolite of DDI, in the cell (Ahluwalia et al., 1993). The effectiveness of DDI by the oral route, however, was less effective than that by the subcutaneous route, suggesting that DDI might be inactivated by acid in the stomach (Anderson et al., 1988). DDC was also about 5 times less potent than AZT in the in vitro assay, but more potent than AZT in the mouse/MT-4 assay if given by either oral or subcutaneous route.

We also examined the in vivo efficacy of two non-nucleoside RT inhibitors, HEPT and TIBO. HEPT was quite effective by subcutaneous administration and a little less effective by the oral administration: the ED_{50} by the subcutaneous route and the ED_{50} by the oral route were about 0.9 and 4 mg/kg, respectively. On the other hand, TIBO, even though possessing marked in vitro activity against HIV-1 replication (Table 1), showed little, if any, activity in vivo following oral administration. The blood concentrations of

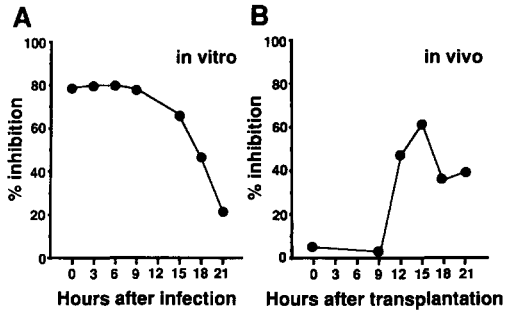

Fig. 6. Time-of-addition effect of KNI-272 treatment on HIV-1 replication in vitro and in vivo. A: HIV-1-infected MT-4 cells were treated with KNI-272 (2 μg/ml) starting at various time points in vitro. At 24 h after infection, RT activity in the supernatants of culture fluids was measured. Data are mean values of duplicate cultures. B: KNI-272 (250 mg/kg) was administered subcutaneously at various time points to SCID mice transplanted with HIV-1-infected MT-4 cells. At 24 h after transplantation, RT activity in the peritoneal wash fluids was determined. Data are mean values of 3 mice.

TIBO following oral administration were also quite low compared to those of AZT (Table 3). The low efficacy of TIBO in vivo is thus probably due to its low bioavailability (De Wit et al., 1992). The bioavailability of HEPT also appeared to be as low as that of TIBO (Table 3), but HEPT was still effective in the mouse/MT-4 assay probably because HEPT is about 100 times more potent in anti-HIV-1 activity than TIBO (Table 1).

3.5. Evaluation of viral protease inhibitors in the mouse / MT-4 assay

The protease encoded by HIV-1 is essential for the processing of HIV-1 gene products, and is regarded as a good target for development of new anti-HIV-1 drugs. We next examined the efficacy of 3 inhibitors of HIV-1 protease in our mouse/MT-4 assay. We first determined the timing effect of KNI-272 (Mimoto et al., 1992) on in vitro HIV-1 replication (Fig. 6A). In sharp contrast to AZT that had to be added early after HIV-1 infection (Fig. 4A), KNI-272 was capable of inhibiting HIV-1 replication even when added as late as 9 h after HIV-1 infection. This is consistent with the role of HIV-1 protease in the late stage of HIV-1 replication. We next examined the anti-HIV-1 effect of KNI-272 in the mouse/MT-4 assay. As shown in Fig. 6B, KNI-272 was most effective when administered at about 15 h after transplantation. The blood concentration of KNI-272 following oral administration reached a peak level within 30 min and rapidly declined thereafter (Table 3). This probably accounts for the narrow duration of effective administration time points.

Fig. 7 shows the dose-dependent effects of 3 protease inhibitors administered orally or subcutaneously in the mouse/MT-4 assay. No cytotoxicity was observed with these drugs since the levels of viability of MT-4 cells recovered from treated mice were equal to the viability of MT-4 cells recovered from non-treated mice (data not shown). Both KNI-272 and Ro 31-8959 showed dose-dependent inhibitory effects. Subcutaneous administration was more effective than oral administration for both drugs. The ED_{50}

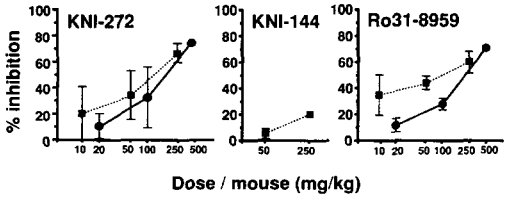

Fig. 7. Effects of various protease inhibitors on HIV-1 replication in MT-4 cells transplanted in mice. KNI-272, KNI-144, and Ro 31-8959 were administered subcutaneously (■) once at 15 h or orally (●) twice at 15 and 18 h after transplantation of HIV-1-infected MT-4. Data are mean values ± S.D. of 3–5 mice for two experiments.

following a single subcutaneous administration at 15 h after transplantation was about 70 mg/kg for KNI-272 and about 50 mg/kg for Ro 31-8959. On the other hand, oral administration had to be repeated twice at 15 and 18 h after transplantation to obtain an ED_{50} of about 130 mg/kg for KNI-272 and about 160 mg/kg for Ro 31-8959. Apparently, the bioavailability of these drugs is much lower by the oral route than by the subcutaneous route. KNI-144, a compound closely related to KNI-272, showed very weak in vivo activity even following subcutaneous administration.

4. Discussion

Animal models are important for preclinical evaluation of potential drugs because of bioavailability, metabolism, clearance, toxicity, etc., that cannot be assessed by in vitro studies. Recently, SCID mice reconstituted with human lymphoid cells (SCID-hu) were introduced as a potential mouse model allowing HIV infection in vivo (McCune et al., 1990; Torbett et al., 1991). However, it is rather impractical to use the SCID-hu system for routine evaluation of potential anti-HIV-1 drugs. The mouse/MT-4 assay described here, although having a number of shortcommings as an animal model, may be useful as an alternative model for preliminary evaluation of in vivo efficacy of a number of anti-HIV compounds. Compared to the SCID-hu mouse model, the mouse/MT-4 assay is advantageous in that it does not use human tissues, does not require maintenance of reconstituted mice, and can be carried out over a short time period. Preliminary data on each compound can be obtained within 3 days. This enables rapid evaluation of candidate compounds and their derivatives for potential in vivo efficacy. Since one dose of the compound suffices, the assay can be carried out with compounds that are limited in supply.

In the mouse/MT-4 assay, viral replication does not go through more than one or two cycles (Fig. 1 and Table 2). Thus, the growth kinetics of HIV-1 in the mouse/MT-4 assay is quite different from natural acute infection of HIV-1. A test compound has to be administered either at a carefully chosen time point depending on its mode of action, as done in the present study, or it may be administered at multiple time points. The efficacy of a compound in this model will be dependent on its effective plasma level, which, in turn, is affected by factors such as bioavailability, metabolism, and clearance. These

factors are in fact critical for the therapeutic success of a potential drug. The mouse/MT-4 assay as described here might be useful for preliminary in vivo evaluation of selected test compounds, and thus facilitate the development of effective anti-HIV drugs.

Acknowledgements

We thank Dr. Shinji Harada for supplying HIV-1(IIIB strain), Dr. Masanori Hayami for M8166 cells and Dr. Yorio Hinuma for his constant support and encouragement.

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