Inhibition of Multidrug-Resistant HIV-1 by Interference with Cellular S-adenosylmethionine Decarboxylase Activity

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S-adenosylmethionine decarboxylase (SAMDC), a key enzyme in polyamine biosynthesis, can be specifically inhibited by the experimental drug SAM486A. The pharmaceutical interference with SAMDC activity results in the depletion of the intracellular pool of spermidine and spermine. In particular, low spermidine levels compromise hypusine modification and, thereby, activation of eukaryotic initiation factor 5A (eIF-5A), which is a cellular cofactor of the essential human immunodeficiency virus type 1 (HIV-1) regulatory protein Rev. In the present study, we show that SAM486A efficiently suppresses HIV-1 replication, including the replication of viruses that are resistant to multiple reverse transcriptase and protease inhibitors. At drug concentrations that efficiently inhibit the formation of progeny viruses, no toxic effects of SAM486A on cellular metabolism are observed. It is demonstrated that the antiretroviral effect of SAM486A is based on the fact that Rev activity is severely compromised in drug-treated cells. Thus, inhibition of cellular SAMDC activity may provide a novel strategy to achieve suppression of otherwise drug-resistant viruses.
Inhibition of HAART-Resistant HIV

Figure 1. Schematic representation of the polyamine and eukaryotic initiation factor 5A (eIF-5A) biosynthesis pathway. Putrescine is formed by decarboxylation of ornithine, a reaction that is catalyzed by ornithine decarboxylase (ODC). Spermidine is synthesized by the action of putrescine aminopropyl transferase (PAPT). The aminopropyl group is derived from decarboxylated S-adenosylmethionine (dc-AdoMet), which is provided by S-adenosylmethionine decarboxylase (SAMDC). The experimental drug SAM486A is a potent inhibitor of SAMDC [3]. Spermine is formed by addition of an aminopropyl group to spermidine by enzymatic action of spermidine aminopropyl transferase (SAPT). The biological activity of eIF-5A depends on its hypusine modification, a spermidine-dependent posttranslational reaction that is catalyzed by the subsequent action of 2 enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DHH).

The cellular spermidine level should indirectly abolish eIF-5A activity by depriving DHS of one of its substrates [8]. Interestingly, it has been demonstrated elsewhere that eIF-5A is an essential cellular cofactor of the Rev trans-activator of HIV-1 [9, 10]. Subsequently, the direct inhibition of DHS or DHH with low-molecular-weight compounds has been shown to profoundly block Rev activity and, in consequence, virus replication [11–13]. Therefore, we hypothesized that inhibition of the polyamine biosynthetic pathway may also provide a potent strategy to interfere with the retroviral life cycle.

In the present study, we analyzed the effect that the specific SAMDC-inhibitor SAM486A has on HIV-1 replication. We show that SAM486A indirectly targets the posttranscriptional Rev pathway and thereby efficiently suppresses the formation of progeny viruses without affecting the metabolism of the host cell.

MATERIALS AND METHODS

Compound. For interference with the polyamine biosynthesis pathway by cellular depletion of spermidine and spermine, the low-molecular-weight SAMDC-inhibitor SAM486A (4-[aminoiminomethyl]-2,3-dihydro-1H-inden-1-diaminomethylenehydrazone) [3] (provided by Novartis Pharma AG Basel), also named “Sardomozide” or “CGP 48664,” was used.

Analysis of cellular toxicity. Cell viability was analyzed by measuring cellular metabolic activity using the alamarBlue redox indicator (Seteroc), in accordance with the manufacturer’s protocol. For cell-cycle analysis, PM1 cells (provided by M. Reitz, AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]) were cultured for 7 days in the presence of 0.4 µmol/L SAM486A or in medium alone (control). Flow cytometry (FACS) analysis was performed using DNA staining with propidiumiodide (CycleTest Plus; Becton Dickinson). For analysis of apoptosis, peripheral-blood mononuclear cells (PBMCs) from a healthy donor were cultured in 0.4 µmol/L SAM486A or medium alone (control) for 12 days. Subsequently, apoptotic cells were assayed by FACS analysis with fluorescein isothiocyanate–coupled annexin V (Bender MedSystems).

HIV-1 infection experiments. HIV-1 infection using the CCR5-tropic strain HIV-1Ba-L (provided by S. Gartner, M. Popovic, and R. Gallo, AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) [14] or the multidrug-resistant (MDR) CXCR4-tropic HIV-1 isolate FE9 [11] was routinely performed in PM1 cells. The drug-resistance–conferring mutations in the FE9 isolate and the respective phenotypic drug sensitivity profile have been described elsewhere [11].

PM1 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum (Pansystems GmbH) and antibiotics (pen-
icillin and streptomycin) and were infected with the virus strains exactly as described elsewhere [11]. After infection, cells were washed twice with PBS without Ca\(^2\+) and Mg\(^2\+) to avoid false-positive p24 antigen determination. Cells were resuspended, and identical aliquots of 5 \times 10^3 infected cells/mL were further cultured in RPMI 1640 medium in the presence of SAM486A or medium (solution control) at various concentrations, for the calculation of the inhibition of virus replication. Culture medium was changed, and cells were split 1:1 at the various days after infection (as indicated in the figures). At the same time, cell viability (measured by alamarBlue assay) and p24 antigen levels in the supernatant (Innogenetics NV) were determined. In addition, total cellular protein extracts were subjected to Western blot analyses with the HIV-1 p24 monoclonal antibody (MAB) 183-H12-5C (provided by B. Chesebro and K. Wehrly, AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) [15] or an \(\alpha\)-tubulin-specific (protein-loading control) MAB (Sigma-Aldrich).

**Analysis of de novo infection and proviral integration.** De novo infection was examined by polymerase chain reaction (PCR) of extrachromosomal circular viral DNA. For this, PM1 cells were cultured for 10 days in the presence of 0.4 \(\mu\)mol/L SAM486A or medium alone (control) and were subsequently infected with HIV-1 \(_{\text{inu}}\) \(\times 1\). Total genomic DNA was isolated from uninfected cells and from the infected cells at 90, 120, and 150 min after infection; 3.0 \(\mu\)g of these genomic DNAs were directly amplified using HIV-1-specific primer pairs recognizing extrachromosomal 1–long terminal repeat (LTR) and 2-LTR circular preintegration DNA (PID), as described elsewhere [16]. Detection of integrated proviral DNA was assayed at day 3 after infection by nested PCR with 2.5 \(\mu\)g of genomic DNA and primer pairs complementary to the HIV-1 \(_{\text{inu}}\) LTR and chromosomal Alu repeats: LTR outer/forward, 5'–GACGAGTA-TCTCAGAGCCTTG–3'; human Alu outer/reverse, 5'–TGCTG-AGATCAGGGCGT–3'; LTR nested/forward, 5'–GACGA-ATCACAACTAGTACAACTACG–3'; and LTR nested/reverse, 5'–CCTTGTAGAAGGCTCAGT–3'. The amplification profile involved 30 cycles of denaturation at 95°C for 1 min, primer annealing at 54°C for 1 min, and primer extension at 72°C for 3 min for the first round of PCR, followed by 40 cycles for the nested PCR.

**Two-dimensional gel electrophoresis and immunoprecipitation analysis.** To analyze the effects that the SAMDC-inhibitor SAM486A has on hpyusine modification of eIF-5A by 2-dimensional gel electrophoresis, 6 \times 10^6 PM1 cells were metabolically labeled for 12 h using 25-\(\mu\)Ci [1,4-\(\text{14C}\)] putrescine dihydrochloride (metabolic precursor of spermidine; 107 mCi/ mmol; Amersham) in the presence or absence of 0.4 \(\mu\)mol/L SAM486A. Gel analysis was performed by autoradiography, as described elsewhere [11]. For immunoprecipitation of eIF-5A, 2 \times 10^6 HeLaCD4-CAT cells (provided by B. K. Felber and G. N. Pavlakis, AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) [17] were metabolically labeled as described above. The various cellular extracts were prepared and analyzed using a rabbit polyclonal anti–eIF-5A antibody, as described elsewhere in detail [11].

**Transfection experiments and plasmids.** HeLaCD4-CAT cells were transiently transfected using Lipofectamine Plus reagent (Invitrogen), in accordance with the manufacturer’s protocol. Rev trans-activation was investigated by cotransfection of 2.5 \times 10^5 HeLaCD4-CAT cells with 0.6 \(\mu\)g of pCMVgag-LucRRE [18] and 0.3 \(\mu\)g of pBC12/CMV/\(\beta\)-gal DNA [19], together with 0.3 \(\mu\)g of either pcRev [20] or the parental pBC12/CMV plasmid. At 48 h after transfection, cellular lysates were prepared, and the levels of \(\beta\)-galactosidase (\(\beta\)-gal; transfection-efficiency control) and luciferase were assayed by ELISA (Roche Applied Science) or luminescence (Promega), respectively. Likewise, Tat trans-activation was investigated by cotransfection of 2.5 \times 10^5 HeLaCD4-CAT cells with 1.0 \(\mu\)g of pBC12/HIV/CAT DNA [21] and 0.6 \(\mu\)g of pBC12/CMV/\(\beta\)-gal DNA, together with 0.5 \(\mu\)g of either pcTat [20] or the parental pBC12/CMV plasmid. At 48 h after transfection, cellular lysates were prepared, and the levels of \(\beta\)-gal (transfection-efficiency control) and chloramphenicol acetyltransferase (CAT) were assayed by ELISA (Roche Applied Science). To analyze HIV-1 gag-pol mRNA export in SAM486A-treated and untreated HeLaCD4-CAT cells, the subgenomic vectors GPV-RRE and GPV-4×CTE (provided by M. H. Malim, King’s College London, UK) were used [22]. For this, 2.5 \times 10^5 cells were cotransfected with either 0.5 \(\mu\)g GPV-RRE or 0.5 \(\mu\)g of GPV-4×CTE together with 0.25 \(\mu\)g of pBC12/CMV/SEAP (transfection-efficiency control) [21] and 0.25 \(\mu\)g of either pcRev or the parental pBC12/CMV plasmid. At 48 h after transfection, cell supernatants were assayed for secreted alkaline phosphatase (SEAP) [21] and p24 antigen.

**RESULTS**

To specifically interfere with the polyamine biosynthesis pathway, in the present study, we employed SAM486A, a well-established and potent low-molecular-weight inhibitor (IC_{50} 5 nmol/L) of SAMDC [3]. This compound has been reported to inhibit the growth of various tumor cell lines and also to induce potent antitumor activities in vivo, particularly in melanoma models [3, 23–26]. SAM486A is, therefore, currently being tested in various phase 1 and phase 2 clinical trials in patients with cancer [27–31].

**Effects that SAM486A has on cellular metabolism.** SAMDC provides the activated precursor dc-AdoMet for spermidine and spermine formation (figure 1) and is a rate-limiting enzyme in cellular polyamine biosynthesis [5]. Because polyamines are involved in multiple cellular processes [1], attempts to interfere with SAMDC activity may also induce general toxic effects on cellular metabolism. Therefore, we first analyzed the
effects that various concentrations of SAM486A have on cellular metabolic activity. For this, PM1 cells were incubated for 17 days in the presence of increasing concentrations (0.1–0.8 μmol/L) of SAM486A or in medium alone (control) and were subsequently analyzed by a cytotoxicity assay with alamarBlue. As shown, SAM486A did not induce any detectable toxic effect on cellular viability up to a drug concentration of 0.4 μmol/L (figure 2A). Therefore, this dose was chosen as the standard SAM486A concentration in the following experiments. Next, cell-cycle progression was analyzed in PM1 cells that were exposed for 7 days to SAM486A (figure 2B). In addition, apoptosis was examined in PBMCs, which were, before analysis, cultured for a period of 12 days in the presence of the SAMDC inhibitor (figure 2C). These analyses demonstrated that a concentration of 0.4 μmol/L SAM486A did not negatively affect cell-cycle progression or cellular apoptosis. The exposure of the cells to

Figure 2. Analysis of cellular toxicity. A, Cell viability assay. PM1 cells were cultured for 17 days in various concentrations of SAM486A or in medium alone (control). Cellular metabolic activity was tested by alamarBlue assay (Serotec) at the indicated days. B, Cell-cycle analysis. PM1 cells were cultured for 7 days in the presence of 0.4 μmol/L SAM486A or in medium alone (control). Flow cytometry (FACS) analysis was performed by DNA staining with propidium iodide. C, Apoptosis assay. Peripheral-blood mononuclear cells from a healthy donor were cultured for 12 days in the presence of 0.4 μmol/L SAM486A or in medium alone (control). Subsequently, apoptotic cells were assayed using FACS analysis with fluorescein isothiocyanate–coupled annexin V. LL, lower left; LR, lower right; UL, upper left; UR, upper right.
this drug concentration did obviously not cause deleterious effects on host cell metabolism and, hence, appears to be well tolerated in our cellular systems.

**SAM486A inhibition of HIV-1 replication.** We next performed HIV-1 infection experiments to elucidate the potential antiretroviral effect of SAM486A. To determine inhibition of virus replication, PM1 cells were infected with the CCR5-tropic stain (R5) HIV-1 BaL [14]. After infection, cells were washed, resuspended, and cultured in the presence of various concentrations of SAM486A or in medium alone (control). The culture medium was changed, and cells were split 1:1 every third day.

Cell viability and p24 antigen levels in the supernatants were determined at days 6 and 9 after infection. As shown, SAM486A displayed pronounced antiretroviral properties in the drug-treated cell cultures. In fact, the rate of inhibition of virus replication was ≥98% in the cell cultures supplemented with 0.2 or 0.4 μmol/L SAM486A, compared with that in the untreated control cultures (figure 3A, top). Cell viability assays, which were performed in parallel, indicated again that no adverse effects on cellular metabolism were elicited at the tested drug concentrations (figure 3A, bottom). Moreover, total cellular protein extracts from a drug-treated culture (0.4 μmol/L...
SAM486A) and the control culture were prepared at day 6 after infection and subjected to HIV-1 p24–specific and α-tubulin–specific Western blot analysis. As shown, the expression of both the p55Gag and p24Gag viral structure proteins were clearly reduced in SAM486A-treated cells, compared with that in the control cells (figure 3B). The detection of extrachromosomal circular viral DNA by PCR analysis [16] revealed that inhibition of SAMDC did not interfere with de novo infection of the host cell (figure 3C). This is in agreement with the comparable levels of integrated proviral DNA in drug-treated cells as well as in untreated (control) cells at day 3 after infection (figure 3D).

We next wanted to examine whether SAM486A is also able to inhibit replication of a CXCR4-tropic (X4) virus isolate that is unresponsive to the full array of reverse transcriptase and protease inhibitors. The genotype and the antiretroviral drug profile of the respective MDR (i.e., highly active antiretroviral therapy [HAART]–resistant) FE9 isolate, as determined by DNA sequencing and phenotypic resistance testing by use of the recombinant virus technique [32], has been presented in detail elsewhere [11]. Infection experiments, which were performed over a period of 18 days, demonstrated that virus replication of this MDR isolate was indeed dramatically reduced in cultures that were supplemented with 0.4 μmol/L SAM486A, compared with that in the untreated control culture. In fact, virus inhibition rates were obtained in these experiments that ranged from 94%–99% (figure 4A, top). The direct monitoring of the p24 antigen levels revealed minimal amounts of viral antigen in the supernatants of SAM486A-treated cells, com-

![Figure 4](image-url)

**Figure 4.** Inhibition of a multiple highly active antiretroviral therapy–resistant HIV-1 isolate. A, PM1 cells, infected with an antiretroviral drug–resistant virus and cultured in the presence of 0.4 μmol/L SAM486A or in medium alone (control). Culture medium was changed every third day, and cells were split 1:1. Cell viabilities and p24 antigen levels were determined at the indicated days. The percentage of inhibition of virus replication in the drug-treated culture, compared with replication in the untreated control culture, is shown at top. The respective virus growth curves as measured by p24 antigen levels in the culture supernatants are shown at bottom. B, Cellular toxicities, determined in the same cultures by alamarBlue assay (Serotec) for metabolic activity. NA, not applicable.
pared with that in the untreated cultures (figure 4A, bottom). The analysis of the cell viabilities demonstrated that the vigorous virus replication starting at day 15 after infection caused death in the unprotected cell culture, whereas, in sharp contrast, SAM486A-treated cells remained viable (figure 4B).

**Inhibition of hypusine modification of eIF-5A by SAM486A.**

The essential nature of spermidine for hypusine modification of the eIF-5A has been well established [8]. This posttranslational modification converts an inactive eIF-5A precursor into an active hypusine-containing form (figure 1). To analyze the effect that SAMDC inhibition has on hypusine modification of eIF-5A, we metabolically labeled PM1 and HeLaCD4-CAT cells by using [14C]putrescine in the presence or absence (control) of 0.4 μmol/L SAM486A. The 2-dimensional gel electrophoresis of the corresponding total PM1 extracts and subsequent autoradiography of the respective gel revealed an almost complete inhibition of eIF-5A hypusine modification in the SAM486A-treated cells (figure 5A, top), whereas, in contrast, the unique hypusine-containing protein eIF-5A and its known isoforms [33] were easily detectable in cells not exposed to SAM486A. These data were confirmed by eIF-5A–specific immunoprecipitation analysis of the radiolabeled protein extracts obtained from the HeLaCD4-CAT cells (figure 5B).

**Specific inhibition of the HIV Rev pathway by blocking of SAMDC.** Because the hypusine-containing protein has been described elsewhere to be an essential cellular cofactor of the HIV-1 regulatory protein Rev [9, 10], we next wanted to investigate whether inhibition of SAMDC by SAM486A specifically interferes with Rev activity. Because eIF-5A is a protein with a long half-life in mammalian cells (>24 h) [34], we first cultured HeLaCD4-CAT cells for 5 days in the presence or absence of 0.4 μmol/L SAM486A. Then, the respective SAM486A-treated and untreated cell monolayers were transiently cotransfected with the standard Rev- and Tat-responsive reporter vectors pCMVgagLucRRE or pBC12/HIV/CAT (figure 6A and 6B, respectively) [18, 21] and plasmids expressing the matching retroviral trans-activator proteins. As shown, SAM486A clearly inhibited Rev trans-activation, reaching an inhibition rate of ∼72% in this assay (figure 6A), whereas no significant negative effect on Tat-mediated transcriptional activation of the HIV-1 LTR promoter was observed (figure 6B). This finding suggested that SAM486A indeed represses HIV-1 replication by interfering with the posttranscriptional Rev-mediated processing of retroviral mRNAs.

Rev is an essential HIV regulatory protein that primarily mediates the nucleocytoplasmic translocation of incompletely spliced and unspliced gag-pol and env mRNAs but also affects the half-life and translation of these mRNAs (reviewed in [35, 36]). Interestingly, a recent study has demonstrated, in murine cells, that the cytoplasmic fate of retroviral mRNAs is determined in the cell nucleus, depending on which distinct cellular nuclear export pathway is utilized [22]. This has been elegantly demonstrated by employing subgenomic HIV-1 gag-pol vectors that solely differ in their cis-active RNA transport signals (figure 7A). The GPV-RRE construct contains the homologous HIV-1 RNA Rev response element (RRE), which binds to Rev and thereby commits this transcript to the specific CRM1/exportin 1 nuclear export pathway (for details on nuclear export pathways, see Rodriguez et al. [37]). In contrast, the GPV-4×CTE vector contains 4 tandem copies of the heterologous constitutive transport element (CTE) of Mason-Pfizer monkey virus [38], which assigns the respective transcript to the NXF1/TAP pathway, the pathway that commonly handles the nuclear export of the bulk of cellular mRNAs [39]. Thus, compounds that negatively affect Gag expression from the GPV-RRE vector, but not from the GPV-4×CTE construct, are highly specific inhibitors of the HIV Rev pathway.

To test this conclusion, we again cotransfected monolayers of SAM486A-treated (0.4 μmol/L) and untreated HeLaCD4-CAT cells in various combinations with the Rev-responsive con-
Figure 6. Effect that SAM486A has on HIV-1 trans-activation. A, Analysis of Rev trans-activation, by use of the Rev-responsive pCMVgagLucRRE reporter plasmid. HeLaCD4-CAT cells were cultured for 5 days in 0.4 μmol/L SAM486A or medium alone (control) and were subsequently cotransfected with pCMVgagLucRRE, a Rev-expressing vector (+Rev) or the respective parental plasmid (Rev-deficient control [−Rev]), and the constitutive internal control vector pBC12/CMV/β-gal. Rev activity was determined at 48 h after transfection by luciferase reporter assay. All luciferase values were adjusted for transfection efficiency by determining the level of β-galactosidase (β-gal) in each culture. B, Analysis of Tat trans-activation, by use of the Tat-responsive pBC12/HIV/CAT reporter construct. HeLaCD4-CAT cells were cultured in SAM486A as described above and subsequently cotransfected with pBC12/HIV/CAT, a Tat-expressing vector (+Tat) or the parental plasmid (Tat-deficient control [−Tat]), and the internal control vector pBC12/CMV/β-gal. Tat activity was determined at 48 h after transfection by chloramphenicol acetyltransferase (CAT) detection. All CAT values were adjusted for transfection efficiency by determining the level of β-gal in each culture. CMV-IE, cytomegalovirus immediate early promoter; HIV-LTR, HIV–long terminal repeat promoter; pA, polyadenylation site; RRE, Rev response element; SA, splice acceptor; SD, splice donor.

struct GPV-RRE; the Rev-independent vector GPV-4×CTE, a Rev-expressing vector; or the respective parental plasmid (Rev-deficient control) and a constitutive internal (transfection efficiency) control vector expressing SEAP. Culture supernatants were assayed for p24Gag and SEAP expression at 48 h after transfection. As expected and shown in figure 7B, in the absence of Rev, no p24Gag was detectable in the supernatants of the GPV-RRE–transfected cultures, irrespective of whether SAM486A was present. Clearly, the cotransfection of a Rev expression plasmid resulted in the detection of pronounced p24Gag levels, revealing ongoing Rev trans-activation; most important, this activity was significantly suppressed in cells exposed to SAM486A. Moreover, expression of p24Gag from the GPV-4×CTE vector was fully resistant to SAMDC inhibition. These data were also reflected by the Western blot analysis of p55Gag protein synthesis in respective cellular extracts (figure...
Figure 7. Specific inhibition of the HIV-1 Rev pathway by SAM486A. A, Schematic representation of HIV-derived subgenomic gag-pol constructs [22]. Transcripts expressed from GPV-RRE access the CRM1/exportin 1 nuclear export pathway. mRNAs expressed from GPV-4×CTE are transported across the nuclear envelope via the NXF1/TAP pathway. B, HeLaCD4-CAT cells, cultured for 5 days in 0.4 μmol/L SAM486A or medium alone (control), followed by cotransfection with GVP-RRE and a Rev-expressing vector (+Rev) or with the respective parental plasmid as a Rev-deficient control (−Rev) and a constitutive internal control vector, pBC12/CMV/SEAP. Likewise, cultures were transfected with GVP-4×CTE, a negative control plasmid (to maintain input DNA levels) and the constitutive internal control vector, pBC12/CMV/SEAP. At 48 h after transfection, cell supernatants were assayed for secreted alkaline phosphatase (SEAP) and p24 antigen levels. In addition, p55 Gag- and α-Tubulin-specific (protein-loading control) Western blot analyses were performed from representative cell cultures at day 6 after infection. CMV-IE, cytomegalovirus immediate early promoter; CTE, constitutive transport element; pA, polyadenylation site; RRE, Rev response element; SA, splice acceptor; SD, splice donor.

Taken together, these data demonstrated that the experimental drug SAM486A indirectly inhibits the HIV-1 Rev pathway.

DISCUSSION

Since its introduction into clinical practice in the 1990s, HAART has significantly improved morbidity and mortality in HIV-infected patients [40]. Currently, HAART targets the viral proteins reverse transcriptase, protease, and gp41. Unfortunately, long-term HAART is often accompanied by substantial toxic side effects in patients [41]. Moreover, HIV is capable of developing high-level resistance to all known inhibitors that are used in the present HAART regimens, and the transmission of drug-resistant strains is being increasingly reported (reviewed in [42]). Therefore, there is a need for advanced antiretroviral strategies that provide novel therapy options, particularly for patients infected with MDR HIV strains. A conceivable approach to developing such a salvage strategy may be the pharmaceutical targeting of cellular enzymes, whose activities are required for virus replication.

In the present study, we have demonstrated antiretroviral activity of the low-molecular-weight drug SAM486A, which has been identified elsewhere to be an efficient inhibitor of cellular SAMDC [3, 43, 44]. SAMDC provides decarboxylated S-adenosylmethionine (dc-AdoMet) (figure 1) for synthesis of spermidine and spermine [5, 45] and is, therefore, a central enzyme of the polyamine biosynthesis pathway [46]. It has been suggested elsewhere that compounds interfering with polyamine biosynthesis and/or function may have considerable potential for use as therapeutic agents, particularly for treatment of tumors and diseases caused by infection with parasites or HIV [5, 47, 48]. In fact, SAM486A is currently being tested in phase
2 clinical studies for treatment of metastatic melanoma and refractory or relapsed non-Hodgkin lymphoma [30, 31].

In the present study, we were able to demonstrate pronounced antiviral effects in SAM486A-treated cultures (EC_{50} 
~0.1 µmol/L), which were infected with the slow-replicating, CCR5-tropic laboratory strain HIV-1_{3a.6L} (figure 3). Likewise, the formation of progeny viruses was also inhibited by SAM486A when the rapidly replicating CXCR4-tropic virus isolate FE9, which is characterized by high-level resistance to the full array of reverse transcriptase and protease inhibitors [11], was analyzed (figure 4). It is important to note that this virus inhibition was not due to general drug-induced cellular toxicity, because the applied SAM486A concentration (0.4 µmol/L) perfectly blocked virus replication but did not induce deleterious effects on cell-cycle progression, cell death, and cellular metabolism (figure 2). Interestingly, in the phase 2 clinical studies mentioned above, patients were treated with 100 mg/m² SAM486A [30, 31], which corresponds to an approximate drug concentration of >100 µmol/L, well above the concentration that demonstrated pronounced anti-HIV activity in the present study. This suggests that inclusion of low-dose SAM486A regimens in future anti-HIV therapies may indeed be tolerable. Clearly, additional preclinical and clinical analyses are necessary before such an approach may become a therapeutic reality. Nevertheless, our findings further support the notion that the targeting of host-specific components may provide a strategy to block replication of otherwise MDR (i.e., HAART-resistant) viruses.

The antiretroviral property of SAM486A can be explained by the fact that inhibition of SAMDC indirectly interferes with hypusine modification and, thereby, activation of eIF-5A. Hypusine is unique in eIF-5A and is synthesized by the enzymes DHS and DHH from spermidine (figure 1) (see the references in Park et al. [8]). Thus, activation of eIF-5A strongly depends on the intracellular spermidine pool, which is severely compromised on SAMDC inhibition [3]. Because hypusine-containing eIF-5A is a cellular cofactor of the essential HIV-1 regulatory protein Rev [9,10], decreased spermidine levels are expected to limit Rev activity and, in consequence, reduce the rate of virus replication. Indeed, an anti-HIV strategy targeting Rev activity appears to be conceivable, because it has been reported in previous studies that the direct inhibition of the eIF-5A-modifying enzymes DHS and DHH by low-molecular-weight compounds and/or RNA interference (RNAi) effectively blocked Rev trans-activation and virus formation [11–13].

Although the exact in vivo function of eIF-5A is still unknown, it has been reported that this protein is actively exported from the nucleus to the cytoplasm [49, 50]. Therefore, it appears that, with respect to HIV-1 Rev function, the hypusine-containing protein eIF-5A is part of the Rev-containing ribonucleoprotein complex that translocates retroviral mRNAs, such as the gag-pol transcript, via the export receptor CRM1/exportin 1 from the nucleus to the cytoplasm. This is in agreement with the previous findings that inhibition of the eIF-5A-activating enzyme DHS negatively affects the cytoplasmic accumulation of Rev-regulated retroviral transcripts [11, 12]. For that matter, we were able to demonstrate, using the subgenomic HIV-1–derived vectors GPV-RRE and GPV-4×CTE [22], that Gag expression is fully sensitive to SAM486A treatment when the gag-pol transcript is exported via the CRM1/exportin 1 pathway but is resistant to SAMDC inhibition when the gag-pol mRNA is redirected toward the NXF1/TAP export pathway. In sum, our data suggest that inhibitors of SAMDC specifically restrict Rev function and may be useful for the development of novel antiretroviral therapies.

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References


