S-Adenosylhomocysteine Hydrolase Inhibitors Interfere with the Replication of Human Immunodeficiency Virus Type 1 through Inhibition of the LTR Transactivation

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SUMMARY

Various analogues of adenosine have been described as inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase, and some of these AdoHcy hydrolase inhibitors (e.g., 3-deazaadenosine, 3-dezaaisteromycin, and 3-deazaneplanocin A) have also been reported to inhibit the replication of human immunodeficiency virus type 1 (HIV-1). When evaluated against HIV-1 replication in MT-4 cells, macrophages, or phytohemagglutinin-stimulated peripheral blood lymphocytes infected acutely or chronically with HIV-1IB or HIVgal strains, a wide range of adenosine analogues did not inhibit HIV-1IB replication for 50% at subtoxic concentrations. However, they inhibited HIV-1 replication in HeLa CD4+ LTR-LacZ cells at concentrations well below cytotoxicity threshold. A close correlation was found among the inhibitory effect of the compounds on AdoHcy hydrolase activity, their inhibition of HIV-1 replication in Hela CD4+ LTR-LacZ cells, and their inhibition of the HIV-1 Tat-dependent and -independent transactivation of the long terminal repeat, whereas no inhibitory effect was seen on HIV-1 reverse transcription or a Tat-independent cytemegalovirus promoter. Our results suggest that AdoHcy hydrolase and the associated S-adenosylmethionine-dependent methylation mechanism play a role in the process of long terminal repeat transactivation and, hence, HIV replication.

Various analogues of adenosine have been described as inhibitors of AdoHcy hydrolase. AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to adenosine and L-Hcy (1). Inhibition of AdoHcy hydrolase by adenosine analogues results in an accumulation of AdoHcy, a product inhibitor of S-adenosylmethionine-dependent methylation reactions that use AdoMet as methyl donor. Methylation of DNA, RNA, proteins, and phospholipids play a crucial role in numerous biological processes (for a review, see Ref. 2). Methylation of eukaryotic and viral mRNA (5`-capping) is essential for the maturation of the mRNA and the translation to proteins and hence plays an important role in the virus replicative cycle (2).

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ABBREVIATIONS: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; β-Gal, β-galactosidase; CC50, 50% cytotoxic concentration; C-c2Ado, carbocyclic 3-deazaadenosine; DHCaA, 9-(trans-2, 3-dihydroxycyclopentyl)-adenine; c2DHCaA, 9-(trans-2, 3-dihydroxycyclopentyl)-7-deazaadenine; DHrPA, 9-(2,3-dihydroxypropyl)-adenine; Hcy, homocysteine; HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; NKO-6–16-3, 3-deaza-3-fluoro-adenosine, NOM-14–6-1, 3-deaza-3-chloro-adenosine, NPA, neplanocin A; c3NPA, 3-deazaneplanocin A; PBL, peripheral blood lymphocyte; TAR, transactivation response element; TNF-α, tumor necrosis factor-α; RT, reverse transcription.

De Clercq et al. (3–6) described the broad-spectrum antiviral activity of AdoHcy hydrolase inhibitors; they exhibit a marked activity against negative stranded (−)RNA viruses (i.e., Paramyxoviridae, Rhabdoviridae, Arenaviridae), double-stranded (±)-RNA viruses (Reoviridae), and Poxyviridae (for a review, see Ref. 3). Because of the close correlation between the antiviral activity and the affinity for AdoHcy hydrolase (6, 7), it has been postulated that these inhibitors achieve their antiviral action by targeting the AdoHcy hydrolase.

Previous reports have described the anti-HIV activity of 3-deazaadenosine, an AdoHcy hydrolase inhibitor (8, 9); when evaluated in MT-4 cells, no IC50 values could be obtained due to toxicity to the host cells (4). Mayers et al. (10) reported the activity of 3-deazaadenosine analogues against 3′-azido-3′-deoxythymidine-resistant strains of HIV-1 in vitro. The mechanism of action of these 3-deazaadenosine analogues as anti-HIV drugs was not explored in detail.
We investigated a series of adenosine analogues that are known to be AdoHcy hydrolase inhibitors for their inhibitory effects on HIV-1 replication, in vitro reverse transcription, and transactivation of the HIV-1 LTR. Replication of HIV requires the function of the virus-encoded Tat protein, which is a strong activator of gene expression directed by the HIV LTR promoter and functions through interaction with the cis-acting TAR (11–17).

We found a close correlation among inhibition of AdoHcy hydrolase, inhibition of HIV-1 transactivation, and inhibition of HIV-1 replication. Our results suggest that AdoHcy hydrolase plays an important role in the activation of the HIV transcription and can be considered as a suitable target for the chemotherapy of HIV infections by AdoHcy hydrolase inhibitors.

Materials and Methods

Compounds. Ro5–3335 (18, 19) was synthesized by Wayne A. Spitze and Frantz Victor at Lilly Research Laboratories (Indianapolis, IN). TNF-α and Frantz Victor at Lilly Research Laboratories (Indianapolis, IN). c3DHCaA were from R. T. Borchardt (Departments of Medical Chemistry and Biochemistry, University of Kansas, Lawrence, KS). NPA, 6-ethyl-NPA, 9-ethyl-NPA, and c3NPA were from J. A. Montgomery (Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, AL). Both (R)- and (S)-DHPA were from A. Holy (Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic). c3NPA was from V. E. Marquez (Laboratory of Pharmacology and Experimental Therapeutics, National Cancer Institute, Bethesda, MD). DHCaA and c3DHCaA were from T. Borchardt (Departments of Medical Chemistry and Biochemistry, University of Kansas, Lawrence, KS). KS605 [(+)-9- cis-4-methoxy-cyclopenten-2-yl]-adenine, KS606 [(-)-9-(trans-2,trans-3-dihydroxy-cis-4-methoxy-cyclopentyl)-adene], c3DHCaA, and 5-noraristeromycin [(+)-N(1R,2S,3R,4R,4aR,9-adenine-9-yl)-1,2,3-cyclopentanol-triol] was from S. W. Schneller (College of Veterinary Medicine, Auburn University, Auburn, AL). The structural formulae of the compounds are presented in Fig. 1.

Inhibition of HIV-1 transactivation. Tat-dependent transactivation was monitored mainly as described previously (19) with the following modifications. HeLa-tat-III cells (HeLa cells expressing HIV-1 transactivator, kindly provided by Dr. C. A. Rosen (20)) were transfected with pHIV LacZ [kindly provided by Dr. J. J. Maio (21)] or pCMVβ (22) plasmid DNA by electroporation with an Eurogentec Genepulser (260 V, 1050 μF, and infinite R). pHIV LacZ contains a LacZ gene driven by the HIV-1 LTR promoter, and pCMVβ expresses the LacZ gene under control of the cytomegalovirus promoter. For the Tat-independent transactivation assay, P4 cells (kindly provided by Dr. P. Charneau (23)) were transfected with pHIV LacZ or pCMVβ plasmid DNA and stimulated with TNF-α (50 ng/ml). The compound Ro5–3335, previously reported as an HIV-1 LTR transactivation inhibitor (18, 19), was used as a reference compound.

The electroporated cells (60 × 10⁴/well) were incubated in microtiter plates for 24 hr in the presence of varying concentrations of the test compounds. Then, medium was removed by gentle aspiration, and the monolayers were washed with PBS. Cells were lysed with 25 μl of 0.5% Nonidet P-40, and β-Gal activity in 20 μl of the cell extracts was quantified by a colorimetric assay as described by Sambrook et al. (24). Then, 5 μl of cell extract was used to determine total protein content according to the Bradford method (BioRad, Hercules, CA). The IC₅₀ value was calculated as being the inhibitor concentration that reduces β-Gal expression by 50%.

Inhibition of AdoHcy hydrolase. AdoHcy hydrolase was purified from murine L929 cells using affinity chromatography, and AdoHcy hydrolase activity was measured in the direction of AdoHcy synthesis using 8-[14C]Ado (Amersham, Buckinghamshire, England) and 2 mM dL-Hcy as substrates, as described previously (7). The kinetic properties of the murine AdoHcy hydrolase enzyme differ only slightly from those of human AdoHcy hydrolase. For the human enzyme, Kₘ values of 1.5–1.6 μM have been reported adenosine, whereas the Kₘ values for AdoHcy vary from 0.75 to 15 μM (25). The Kₘ values of purified enzyme from murine L929 cells are 0.5 μM for adenosine and 4.8 μM for AdoHcy.

Inhibition of HIV-1 replication in MT-4 cells. In MT-4 cells, the anti-HIV-1 activities of the test compounds were determined by measuring virus-induced cytopathogenicity (26). Briefly, MT-4 cells were suspended at 3 × 10⁵ cells/ml and infected with HIV at 100 times the 50% cell culture infective dose/ml. Immediately after infection, 100 μl of the cell suspension was added to each well of a 96-well microtiter plate containing various concentrations of the test compound. After 5 days of incubation at 37°C, the number of viable cells was determined according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method as described previously (27).
Inhibition of HIV-1 replication in persistently HIV-1 infected HUT-78/HIV-1IIIb cells. Persistently infected HUT-78/HIV-1IIIb cells were cultured for 3 days in the presence of various concentrations of test compounds. Anti-HIV-1 activity was determined by monitoring viral p24 antigen expression by enzyme-linked immunosorbent assay (DuPont). The supernatant of the cells incubated in the presence of various concentrations of compound was used to detect virus yield according to the Reed and Muench end point dilution assay (28).

Inhibition of HIV-1 replication in macrophages. Primary macrophages (obtained from blood monocytes) were infected with 300 tissue culture infectious doses of monocytopathic strain HIV-1_RL, and maintained in culture until day 10, when chronic infection is usually established (29). At this time, macrophages were treated with different concentrations of DHCaA and c3DHCaA. Drugs were maintained in culture throughout the whole experiment. Inhibition of virus replication was assessed in the supernatants of macrophages at different time points by a commercially available imunosassay able to detect the presence of HIV p24 (Abbott Laboratories, Pomezia, Italy). Toxicity was assessed by trypan blue exclusion method on macrophages detached from wells 5–11 days after the beginning of the treatment and compared with the number and viability of macrophages found in control untreated (but infected) cells. Details about the experimental procedure are given elsewhere (29, 30).

Inhibition of HIV-1 replication in P4 cells. In P4 cells (HeLa-CD4-LTR-LacZ), the anti-HIV-1 activity of the test compounds was determined by measuring virus-induced β-Gal expression and p24 antigen production. At day 1, the cells were plated in microtiter plates at 2×10^4 cells/well and incubated overnight at 37°C with 5% CO2. At day 0, the medium was removed by gentle aspiration, and cells were infected with an excess of HIV-1Rl for ~1 hr (multiplicity of infection >1). After infection, various concentrations of test compounds were added to the infected cells. Two days after infection virus replication was monitored by measuring β-Gal expression and by measuring β-Gal expression in the cell extracts. β-Gal activity and p24 antigen were measured with the same assays as described for the transactivation assay (24) and the other replication assay, respectively. The IC50 value was calculated as being the inhibitor concentration that reduces β-Gal expression or the p24 production by 50%.

HIV-1 RT assay. The enzymatic activity of recombinant HIV-1 RT was tested using poly(C)/oligo(G) as template/primer and [3H]-labeled dGTP as substrate in an RNA-dependent DNA-polymerase reaction. RT assays were performed in a reaction buffer containing 50 mM Tris-HCl, pH 8.1, 2 mM dithiothreitol, 10 mM MgCl2, 0.01% Triton X-100, 2.5 μM [3H]dGTP, 65 μg/ml poly(C)/oligo(G), 2 mM concentrations of RT enzyme preparations, and appropriate concentrations of the compounds, as described previously (31). The IC50 value was calculated as being the concentration of inhibitor that reduces the RT activity by 50%.

Cellular toxicity. Cellular toxicity of the compounds was measured by the trypan blue exclusion, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide method (27), or total protein content in the cell extracts. The latter was determined according to the Bradford method (BioRad).

Results

Inhibition of HIV-1 transactivation. The β-Gal transactivation assay allowed us to quantify HIV-1 LTR transactivation by Tat in Hela-tat-III cells after transient transfection with pHIVLacZ. Tat-independent transactivation of the HIV-1 LTR by TNF-α was also measured in pHIVLacZ-transfected P4 cells. After Tat-dependent or -independent transactivation of the HIV-1 LTR, the expression of β-Gal activity was increased up to 50-fold; therefore, these assays provide a sensitive way to study HIV-1 LTR activity.

The compound Ro5–3335, previously reported to be a transactivation inhibitor, inhibited the Tat-dependent expression of the LacZ gene at a IC50 value of 0.44 μM, a result that is compatible with previous reports (18, 19). All the AdoHcy hydrolase inhibitors tested were able to inhibit the Tat-dependent β-Gal expression (Table 1). The most potent inhibitors were DHCaA (IC50 = 0.21 μM), NPA (IC50 = 0.23 μM), 5′-noraristeromycin (IC50 = 0.24 μM), c3DHCaA (IC50 = 0.26 μM), and c3NPA (IC50 = 0.30 μM). The (R)-isomer of 6′-methyl-NPA (IC50 = 0.32 μM) was >110-fold more potent than the (S)-isomer (IC50 > 36 μM). The same differential inhibitory activity was observed for (R)- and (S)-isomers of 6′-ethyl-NPA and 6′-ethenyl-NPA. Adenosine analogues that were inactive as AdoHcy hydrolase inhibitors were also inactive against transactivation of the HIV-1 LTR. Similar IC50 values were obtained for the inhibition of the HIV-1 LTR activation by TNF-α (Table 1). None of the compounds were able to inhibit the β-Gal expression in cells transiently transfected with pCMVβ DNA, a plasmid containing the Tat-independent cytomegalovirus promoter driving the LacZ gene.

Inhibition of HIV-1 replication. In the MT-4/3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide HIV-1 replication assay, the anti-HIV-1 activities of c3NPA and C-c3Ado were determined by measuring the inhibition of virus-induced cytopathogenicity (26). At subtoxic concentrations, no inhibition of HIV-induced cytopathogenicity could be found in MT-4 cells (CC50: c3NPA, 0.072 μM; C-c3Ado, 7.1 μM).

Inhibition of replication of HIV-1IIIb in PBLs was measured by quantifying the p24 antigen production in the presence of varying concentrations of test compound. The IC50 value of DHCaA for HIV-1 replication was 0.052 μM; this was ~2-fold higher than the CC50 value (0.023 μM).

When evaluated for their inhibitory effects on HIV-1 replication in persistently infected HUT-78/HIV-1IIIb cells, c3NPA and C-c3Ado at subtoxic concentrations did not reduce HIV-1 progeny yield, as measured by quantification of the p24 core antigen (CC50: c3NPA, 0.38 μM; C-c3Ado, 15 μM).

The activity of DHCaA and c3DHCaA was then assessed in chronically infected primary macrophages. We found preliminarily that the CC50 value of either compound was in the range of 4.6 μM. No detectable activity, at least in term of production of p24 antigen assessed in the supernatants at different time points (i.e., day 1, 3, or 5 after treatment with antiviral drugs), was found in three consecutive experiments. Approximately 90% inhibition of virus production was achieved with 5 mM 282–870, a protease inhibitor provided of potent activity both in acutely and chronically infected cells (32).

Inhibition of HIV-1 replication in P4 cells was measured by quantifying the transactivation of the HIV-1 LTR by viral Tat using the LacZ reporter gene or quantifying p24 antigen production in the culture medium. P4 cells, HeLa-CD4 cells with stably integrated LTR-LacZ, were infected with HIV-1IIIb and treated with the compounds. Dextran sulfate (IC50 = 0.007 μM), 3′-azido-3′-deoxystymidine (IC50 = 3.4×10^-4 μM), and saquinavir (IC50 = 0.82 μM), known as HIV-1 replication inhibitors, all inhibited the LacZ expression and p24 production in infected P4 cells in a dose-dependent manner.
Inhibitory effects of control compounds DS, AZT, Ro5-3335, and Saquinavir and AdoHcy hydrolase inhibitors on HIV-1 LTR replication

<table>
<thead>
<tr>
<th>Inhibition of virus-induced LacZ expression, IC50</th>
<th>Toxicity, CC50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>µM</strong></td>
<td><strong>µM</strong></td>
</tr>
<tr>
<td>DS 5000</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td>AZT</td>
<td>0.00034 ± 0.000022</td>
</tr>
<tr>
<td>Ro5-3335</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>NPA</td>
<td>1.9 ± 2.7</td>
</tr>
<tr>
<td>c5′NPA</td>
<td>0.41 ± 1.5</td>
</tr>
<tr>
<td>DHCAa</td>
<td>1.5 ± 1</td>
</tr>
<tr>
<td>c5′DHCAa</td>
<td>0.29 ± 0.0042</td>
</tr>
<tr>
<td>C-c5′Ado</td>
<td>7.4 ± 5</td>
</tr>
<tr>
<td>6′-(R)-Methyl-NPA</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>6′-(S)-Methyl-NPA</td>
<td>&gt;36</td>
</tr>
<tr>
<td>(S)-DHPA</td>
<td>248 ± 189</td>
</tr>
<tr>
<td>c5′DHCAa</td>
<td>51</td>
</tr>
<tr>
<td>6′-(R)-Ethynyl-NPA</td>
<td>38</td>
</tr>
<tr>
<td>6′-(S)-Ethynyl-NPA</td>
<td>&gt;104</td>
</tr>
<tr>
<td>6′-(R)-Ethynyl-NPA</td>
<td>6.16 ± 3</td>
</tr>
<tr>
<td>6′-(S)-Ethynyl-NPA</td>
<td>57 ± 52</td>
</tr>
<tr>
<td>6′-(R)-Ethyl-NPA</td>
<td>4.6 ± 0.34</td>
</tr>
<tr>
<td>6′-(S)-Ethyl-NPA</td>
<td>67 ± 3.7</td>
</tr>
</tbody>
</table>

a Concentration of the inhibitor required for 50% inhibition of Tat-transactivation expression driven by the HIV-1 LTR in HIV-1-infected CD4+ HeLa cells.

b Concentration of the inhibitor required for 50% inhibition of total protein expression in cell culture.

Data represent mean values of two to four separate experiments, each performed in triplicate.
0.85 with a slope of 3.8) between the inhibitory effects on AdoHcy hydrolase and Tat-transactivation. The correlation coefficient between the inhibitory effects on virus replication in P4 cells and Tat-transactivation was 0.99 with a slope of 0.15 (Fig. 3). When the IC\textsubscript{50} values of the compounds for HIV-1 replication in P4 cells were plotted as a function of their IC\textsubscript{50} values for AdoHcy hydrolase (Fig. 4), linear regression again showed a close linear correlation (linear $r = 0.91$ with a slope of 0.02) between the inhibitory effects on virus replication and AdoHcy hydrolase.

**Discussion**

The HIV Tat protein stimulates transcriptional initiation and elongation through interaction with a cis-acting element located within the HIV LTR, termed the TAR (13–16). The molecular mechanism by which Tat functions is not yet completely understood, but several lines of evidence suggest that transactivation of the Tat protein is mediated by cellular factors: Tat stimulates transactivation in a cell-dependent manner (33), and cellular proteins that bind TAR in vitro have been described (for reviews, see Refs. 34 and 35). The HIV-1 LTR can also be trans-activated by lymphocyte-activating factors such as TNF-α.

It has been clearly established that adenosine analogues are inhibitory to AdoHcy hydrolase (3, 36, 37). More recently, Mayers et al. (10) reported the activity of 3-deazaadenosine analogues against HIV-1. They postulated that the 5'-O-triphosphate of 3-deazaadenosine (38, 39) may act as an inhibitor of viral DNA or RNA synthesis (10). However, the investigators did not measure the inhibitory activity of the 5'-O-triphosphate derivatives of 3-deazaadenosine analogues against HIV-1 RT in a cell-free system to confirm this hypothesis. Moreover, we believe that it is rather unlikely that ribonucleotides are efficiently recognized by DNA polymerases, such as HIV-1 RT, as a substrate or an inhibitor of the enzyme reaction. In fact, all compounds we tested contain both 2'- and 3'-hydroxyl groups (Fig. 1). Moreover, DHCA and c\textsuperscript{3}DHCA cannot be converted in the cell to a triphosphate form at the 5' position (see Fig. 1) and therefore cannot inhibit the HIV-1 RT reaction as chain terminator. Furthermore, in additional experiments, it was determined that DHCA and c\textsuperscript{3}DHCA did not have a direct inhibitory effect on the HIV-1 RT reaction.
Here, we suggest that these adenosine analogues, described as HIV-1 inhibitors by Mayers et al. (10), most probably act through their inhibition of AdoHcy hydrolase. We demonstrate that adenosine analogues that are able to inhibit AdoHcy hydrolase have potent inhibitory effect on the Tat-dependent and TNF-α-induced transactivation of a reporter gene driven by the HIV-1 LTR. The most active agents that emerged were DHCaA, NPA, 5'-noraristeromycin, c6-DHCaA, and 3-deazaaneplanocin A. The fact that similar IC50 values as obtained for the Tat-dependent inhibition were obtained for the Tat-independent inhibition of LTR transactivation means that these adenosine analogues interfere with the HIV-1 LTR transactivation in general through a Tat-independent mechanism. The compounds were tested in different host cells, but no anti-HIV activity could be observed in MT-4 cells or PBLs at subtoxic concentrations. Moreover, in chronically infected macrophages, we found no detectable activity. This was not surprising, however, because previous data, confirmed in this set of experiments, show that infection of virus production in chronically infected macrophages can be achieved only with protease inhibitors and at concentrations ≥10–50-fold higher than those active in chronically infected lymphocytes and ~100–1000-fold greater than those active after de novo infection of lymphocytes. Indeed, Ro5–3335, known as an HIV transactivation inhibitor, is also completely inactive in this system. When tested in CD4+ HeLa cells, all AdoHcy hydrolase inhibitors inhibited HIV-1 LTR replication at subtoxic concentrations (Table 2). Of the two diastereoisomers of 6'-methyl-NPA, the (R)-isomer proved to be much more active than the (S)-isomer. This differential inhibitory activity of the two diastereoisomers was reflected in their inhibitory effect on HIV-1 LTR transactivation, AdoHcy hydrolase activity, and HIV-1 replication in P4 cells (Tables 1 and 2).

AdoHcy hydrolase is functionally linked to AdoMet-dependent methyltransferases (4). When serving as the methyl donor for methyltransferase reactions, AdoMet generates AdoHcy, which is not only a product but also an inhibitor of the methyltransferase reaction. To avoid this inhibitory effect and allow the methyltransferases to proceed, AdoHcy must be removed by AdoHcy hydrolase. The fact that AdoHcy hydrolase inhibitors exert a potent inhibition of Tat-mediated and TNF-α-induced transactivation of the LTR points to the participation of the AdoMet-dependent methyltransferase/AdoHcy hydrolase system in the transactivation process.

We demonstrated a close correlation among the inhibitory effect on AdoHcy hydrolase, HIV replication, and transactivation activity for a wide range of adenosine analogues (Figs. 2–4). A similar correlation was found earlier between their inhibitory effects on AdoHcy hydrolase and their activity against a number of viruses (vaccinia virus, vesicular stomatitis virus, and cytomegalovirus) (6, 7). The strong correlation between AdoHcy hydrolase inhibition and anti-HIV-1 transactivation suggests that the AdoHcy hydrolase inhibitors may interfere with the HIV transactivation process via inhibition of AdoHcy hydrolase (Fig. 2). Furthermore, the close correlation between the inhibition of virus replication and inhibition of Tat-transactivation suggests that the anti-HIV-1 activity of the AdoHcy hydrolase inhibitors is due to inhibition of HIV-1 Tat-transactivation (Fig. 3). The AdoHcy hydrolase inhibitors may inhibit HIV-1 Tat-transactivation through interference with the HIV-1 transcription or with the 5'-capping of the HIV-1 mRNA. The latter is required for the folding of the TAR stem structure, which in turn is required for the effective activation of the LTR. In any case, the mode of action of the AdoHcy hydrolase inhibitors seems to be specifically related to the HIV-1 LTR transactivation because the AdoHcy hydrolase inhibitors tested did not have any effect on the expression of the LacZ gene driven by a Tat-independent cytomegalovirus promoter.

We suggest that the adenosine analogues exert their anti-HIV-1 activity through inhibition of Tat-transactivation via inhibition of AdoHcy hydrolase. The exact molecular mechanism by which the AdoHcy hydrolase inhibitors interfere with the Tat-transactivation of HIV-1 remains to be elucidated.

Due to the high toxicity in PBLs and T cell lines such as MT-4 and HUT-78 cells, these adenosine analogues might not be immediate candidates for clinical trials. However, they are helpful in improving our understanding of the HIV LTR transactivation mechanism, which opens perspectives for future drug design aimed at interfering with the HIV transactivation.

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References

Inhibition of Human Immunodeficiency Virus Transactivation


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