Antiviral Drug Therapy of Filovirus Infections: S-Adenosylhomocysteine Hydrolase Inhibitors Inhibit Ebola Virus In Vitro and in a Lethal Mouse Model

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Ebola (subtype Zaire) viral replication was inhibited in vitro by a series of nine nucleoside analogue inhibitors of S-adenosylhomocysteine hydrolase, an important target for antiviral drug development. Adult BALB/c mice lethally infected with mouse-adapted Ebola virus die 5–7 days after infection. Treatment initiated on day 0 or 1 resulted in dose-dependent protection, with mortality completely prevented at doses ≥0.7 mg/kg every 8 h. There was significant protection (90%) when treatment was begun on day 2, at which time, the liver had an average titer of 3 × 10^6 pfu/g virus and the spleen had 2 × 10^9 pfu/g. Treatment with 2.2 mg/kg initiated on day 3, when the liver had an average titer of 2 × 10^7 pfu/g virus and the spleen had 2 × 10^9 pfu/g, resulted in 40% survival. As reported here, Carbocyclic 3-deazaadenosine is the first compound demonstrated to cure animals from this otherwise lethal Ebola virus infection.

The emerging pathogens Marburg virus and Ebola (EBO) virus cause very severe hemorrhagic fevers and mortality as high as 90%. As stated by Piot et al. [1], “The evolution of disease often seems inexorable and invariable.” First recognized in 1976, EBO outbreaks in Africa have been associated with high rates of morbidity and mortality. Almost nothing is known about the natural histories of these viruses, despite the field investigations (designed to identify risk factors and reservoirs) that were done after each major outbreak. There are no specific treatments for Marburg and EBO viral hemorrhagic fevers. Ribavirin, an antiviral drug used to treat several other hemorrhagic fevers, has no in vitro effect on Marburg and EBO viruses, failed to protect in multiple primate studies, and is unlikely to have any clinical value to human patients [2]. Human convalescent plasma containing antibodies has been used for treatment in the past, despite the lack of coherent clinical or experimental data regarding its use. Equine IgG with high-titer neutralizing antibodies to EBO virus protected guinea pigs and baboons but failed to protect rhesus monkeys infected with an EBO virus isolated from the 1995 Zaire outbreak [3]. Similarly, human interferon was used in 1 patient [4] despite negative experimental evidence of efficacy in a primate model. The development of effective therapy must be a top priority in preparing to deal with subsequent outbreaks.

Antiviral therapy offers the possibility of reducing morbidity, mortality, and transmission by prophylaxis of high-risk contacts. Discovery of an active antiviral compound by random screening requires either screening tens of thousands of compounds, which is impractical in a maximum-containment biosafety level 4 (BSL-4) environment, or considerable luck. Selection of compounds with a higher probability of antiviral activity against filoviruses can be inferred from the activity of the compound against other viruses with similar molecular targets. Molecular targets for drug intervention are steps central to viral replication, whose inhibition selectively blocks viral replication, either because normal cells lack the equivalent target or the cellular analogue of the target is not inhibited within the range of concentrations that inhibits the viral target. The availability of the full-length sequence of EBO [5] has allowed the identification of homologous sequences in other viruses and the determination of potential shared molecular targets. Filoviruses share similar molecular organization with rhabdoviruses and paramyxoviruses but are closer to the family paramyxoviridae, as determined on the basis of several of our observations. Several targets appear to be functionally identical between EBO and Marburg viruses, making it more likely that one drug would inhibit both.

S-adenosylhomocysteine (SAH) hydrolase (EC 3.3.1.1) is a cell-encoded enzyme that is an important intracellular target for antiviral drug development. Inhibitors of the cellular enzyme indirectly inhibit transmethylation reactions by a feedback mechanism. Several transmethylation reactions involved in viral replication are potential targets, including the viral enzyme (guanine-7-)methyltransferase, which transfers a methyl group from S-adenosylmethionine to the 7 position of guanosine residue of 5' capped viral mRNA, a required step for replication of all viruses that cannot initiate mRNA synthesis by the so-called cap-stealing process. Several characterized SAH inhibitors inhibit some DNA viruses (e.g., poxviruses) and negative-
strand RNA viruses, including filoviruses (Marburg and EBO) [6], rhabdoviruses (rabies, vesicular stomatitis virus), and paramyxoviruses (parainfluenza, respiratory syncytial virus) [7].

How can inhibition of SAH hydrolase, a cellular enzyme involved in the metabolic pathway of many intracellular methylation reactions, impart any specificity toward viral replication that would make it a selective antiviral target? One possible explanation, borne out by experimental data, is that qualitative differences exist between the sensitivity to feedback inhibition by the reaction product SAH of viral methyltransferases and that of the host cell–encoded enzyme (reviewed in [8]). This allows for inhibition at concentrations that do not interfere with cellular methylation. The methyltransferase reaction involves transfer of the methyl group from S-adenosylmethionine to an acceptor molecule, resulting in SAH as a reaction product. This compound is a feedback inhibitor of the enzyme and must be removed by the cellular enzyme SAH hydrolase in order for the enzyme to continue effective methylation. Thus, inhibition of SAH hydrolase by a drug effectively shuts down methylation and any steps in viral replication that are dependent upon methylation.

Materials and Methods

**Biologic containment.** Infectious material and animals were handled in maximum-containment BSL-4 facilities at the US Army Medical Research Institute of Infectious Diseases (USAMRIID). Laboratory personnel wore positive-pressure protective suits (ILC; Dover, Frederica, DE) equipped with HEPA filters and supplied with umbilical-fed air.

**Viruses and cells.** The 1976 Mayinga strain of EBO (subtype Zaire [EBO-Z]) virus was passaged once in Vero cells or three times intracerebrally in suckling mice and then once in Vero cells [9]. EBO-Z viruses (1976 and 1995 strains) that had been passaged twice in Vero cells were provided by Peter Jahrling (USAMRIID). The viruses were amplified in Vero E6 cells, and the supernatants were collected to produce stocks of EBO-Z '76 Vp2, EBO-Z '76 M3 Vp2, and EBO-Z '95 Vp3. The derivation and biologic properties of “mouse-adapted virus,” a doubly plaque-purified, ninth mouse–passage virus derived from the 1976 strain of EBO-Z and designated EBO-Z '76 M3 Vp2 M9 GH, is described elsewhere in this supplement [10]. The E6 line of Vero African green monkey kidney cells, Vero C1008 (ATCC CRL 1586); DBS-FrHl (ATCC CL 160); LLC-MK2 (ATCC CCL 7); MRC-5 (ATCC CCL 171); SW13 (ATCC CCL 105); Vero (ATCC CCL 1586); and BALB/3T3 clone A31 (ATCC CCL 163) were propagated in Eagle MEM with Earle's salts, nonessential amino acids, 10% fetal bovine serum (FBS), glutamine, penicillin, and streptomycin; the same were captured on a computer for manipulation and analysis. The formazan reaction product was completely dissolved. The colored MTT formazan by mitochondrial enzymes in the electron transport chain [13]. Previous filtered MTT (0.83 mg/mL final concentration in subcomplete medium) was then added to the plate and incubated for 2 h at 37°C. Medium was then removed, and the insoluble reaction product was solubilized by adding 100 μL of DMSO. The plate was mixed on an orbital shaker until the reaction product was completely dissolved. The colored MTT formazan reaction product was measured at 570 nm with a Vmax ELISA reader ( Molecular Devices, Menlo Park, CA), and data were captured on a computer for manipulation and analysis. The resulting dose-response curves were fitted to a four-parameter logit curve by use of a computer program (SOFMax; Molecular Devices) to determine the IC50, the TC50 (50% toxic concentration), and the therapeutic index, which was calculated as TC50/IC50. Antiviral protection curves were corrected for toxicity, using a previously described toxicity curve [13].

**Drug-screening antiviral-activity assay based on ELISA.** Plates (96-well) of Vero E6 cells (or FRL-103, LLC-MK2, MRC-5, SW-13, or Vero cells) were prepared, treated with drug, and
Results

**Antiviral activity of SAH hydrolase inhibitors.** An antiviral screening program previously identified anti-EBO activity of a known inhibitor of SAH hydrolase [6]. Inhibition of EBO-Z replication in Vero E6 cells by a series of known SAH inhibitors is shown in figure 1. Permissive Vero E6 monolayers were infected with EBO virus at a low MOI such that if viral replication was inhibited by the compound, there would not be significant spread of the virus to other cells in the monolayer and the ELISA-based viral antigen-detection system would not detect increased viral antigen. Ca-c3Ado and c3-Npc A showed inhibitory values against the EBO-Z viruses (1976 and 1995 strains), EBO (subtype Sudan), and Marburg (Musoke strain) virus in cell lines of primate (FRhL, LLC-MK2, MRC-5, SW13, Vero 76, Vero E6) and mouse (BALB/3T3 clone A31) origin (data not shown).

IC50 was not dependent on the assay format or EBO strain, as shown in table 1, wherein three assay formats (ELISA, MTT, and plaque reduction) were compared for 2 virus strains. Npc A and its structural analogues, c3-Npc A, DHCp-Ado, and DHCp-c3Ado, had equivalent antiviral inhibitory activity (IC50 = 2–17 μM) and were among the most active SAH hydrolase inhibitors found on a molar basis. Npc A, based on a cyclopentonyl (Cp) sugar, was quite toxic (TC50 = 60 μM) to cells. The analogue c3-Npc A, lacking a nitrogen at the 3 position of the adenosine ring and not phosphorylated by adenosine kinase, was nontoxic to cells. Because of the lack of a 5′-OH on the sugar, the analogues DHCpAdo and DHCp-c3Ado could not be metabolized to form the toxic nucleotide triphosphate derivative and, therefore, were also nontoxic (TC50 > 1700 μM).

For compounds based on the carbocyclic (Ca) sugar, Ca-Ado (phosphorylated) was toxic, but its derivatives Ca-c3Ado and Me-Ca-c3Ado (not phosphorylated) were nontoxic. DDFA, the only suicide inhibitor (irreversible inactivator) of the enzyme among the compounds tested, was the most effective at completely eliminating viral replication (see below and figure 1). However, the 3.5-log reduction seen with Ca-c3Ado was adequate to treat animals with considerable virus burdens (see below). The possible advantage of complete inhibition of viral replication in disease treatment has not been evaluated to date. Adenosine dialdehyde, a known SAH hydrolase inhibitor, was effective, while the structurally related guanosine dialdehyde, which is not an inhibitor of the enzyme, was not active. Ribavirin was also inactive, as reported previously [16].

**Quantitative determination of reduction in virus yield.** The reduction in virus yield was determined at the time of maximum viral replication for a graded series of concentrations of Ca-c3Ado and compared with DDFA (figure 2). In a dose-dependent manner, Ca-c3Ado (2 μg/mL, 7.6 μM) reduced viral replication by 3 logs. There was no further inhibition, even with a 100-fold increase in drug concentration. DDFA completely inhibited viral replication at ≥32 μg/mL (119 μM). Most other SAH hydrolase inhibitors tested could not completely inhibit viral replication (data not shown).

**Evaluation of prophylactic administration of Ca-c3Ado in mice.** Previous work in a lethal SCID mouse model for EBO infection demonstrated that both Ca-c3Ado and c3-Npc A could inhibit viral replication when administered every 12 h, but the lack of a functional immune system in these animals precluded a realistic evaluation of the compounds (data not shown). Pharmacokinetic and distribution studies [17, 18] in adult BALB/c mice showed that Ca-c3Ado A is less rapidly eliminated in tissues than c3-Npc A. Although the IC50 for Ca-c3Ado was 15-fold higher than for c3-Npc A, the area under the curve, an indication of the amount of compound available, was 100-fold better. Ca-c3Ado had a slightly longer serum half-life than c3-
Figure 1. Inhibition of Ebola virus replication in Vero E6 cells by series of S-adenosylhomocysteine hydrolase inhibitors. Compounds were assayed by microtiter format ELISA/MTT as described in Materials and Methods; values are average of at least duplicate determinations. AVS = antiviral screening identification no.; \( R_1 \) and \( R_2 \) = substituent groups at \( R_1 \) and \( R_2 \) sugar moiety; \( TC_{50} \) = 50% toxic concentration; \( TI \) = therapeutic index; NA = not active. * Not toxic at maximum concentration tested.
Table 1. Comparison of IC50 (\(\mu M\)) and 50% toxic dose (\(\mu M\)) for various drug compounds by various antiviral assay formats.

<table>
<thead>
<tr>
<th>Drug compound</th>
<th>AVS</th>
<th>ELISA</th>
<th>MIT</th>
<th>Plaque reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribavirin</td>
<td>1</td>
<td>&gt;2050</td>
<td>&gt;2050</td>
<td>&gt;2050</td>
</tr>
<tr>
<td>Ca-c3Ado</td>
<td>303</td>
<td>15</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Npc A</td>
<td>1549</td>
<td>4</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>c3Npc A</td>
<td>4275</td>
<td>0.3</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

NOTE. AVS = antiviral screening identification no.; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; CPE = cytopathic effect; Ca-c3Ado = carbocyclic 3-deazaadenosine; Npc A = (\(\sim\)-9-[trans-2', trans-3'-dihydroxy-4'-hydroxymethyl-cyclopent-4'-enyl]-adenine; c3Npc A = (\(\sim\)-9-[trans-2', trans-3'-dihydroxy-4'-hydroxymethyl-cyclopent-4'-enyl]-3-deazaadenine.

Npc A (23 vs. 13 min) [17]. Adult BALB/c mice infected with 30 LD50 of mouse-adapted EBO were treated prophylactically with Ca-c3Ado every 8 h, beginning 24 h before infection, with doses ranging from 0.03 to 20 mg/kg. A dose-dependent increase in mean time to death was seen as doses increased from 0.08 to 0.3 mg/kg (table 2). Mice were completely protected by doses of \(\geq 0.7\) mg/kg. Drug was well tolerated at the highest dose tested (20 mg/kg), which was 28 times the minimum protective dose. Some weight loss occurred during compound dosing (days -1 to 8), but at doses <2.2 mg/kg, animals returned to baseline weights during the subsequent 5 days. At and above this dose, a less dramatic weight recovery was seen, but all animals remained healthy throughout the study period. Infected, treated animals that survived were rechallenged with 100 pfu (3000 LD50) of the same virus to obtain positive evidence that they were infected in the experiment. All animals were protected, including animals in the higher-dose groups that never showed signs suggesting viral infection (i.e., weight loss greater than toxicity controls).

To determine the latest time that Ca-c3Ado treatment could be successfully initiated, we infected mice as above, and treatment at three dose levels was initiated on days 0, 1, 2, or 3 relative to virus challenge (see below; figure 3). Complete protection occurred when treatment was initiated immediately and after 1 day of infection. Mice were significantly protected (90%) when treatment was begun on day 2. Other experiments showed that the liver had an average titer of \(3 \times 10^5\) pfu/g virus and the spleen had \(2 \times 10^6\) pfu/g. While controls died within a mean of 6.7 days, the only treated animal that died did so on day 15. Treatment (2.2 mg/kg) for 10 mice was initiated on day 3, when the liver had an average titer of \(2 \times 10^7\) pfu/g virus and the spleen had \(2 \times 10^8\) pfu/g virus; 4 of the 10 mice survived. Toxicity, as judged by weight loss greater than that for untreated controls, was seen at the 20 mg/kg dose.
Table 2. Effect of treatment with carbocyclic 3-deazaadenosine (Ca-c3Ado) on survival of 6-week-old BALB/c mice infected with 30 LD_{50} of Ebola virus.

<table>
<thead>
<tr>
<th>Day treatment begun</th>
<th>Dose (mg/kg) every 8 h for 9 days</th>
<th>% change in weight, days 0–1 to 8</th>
<th>% change in weight, days 0–1 to 13</th>
<th>No. survived/total no.</th>
<th>Toxicity</th>
<th>Antiviral efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1</td>
<td>20.0</td>
<td>−16.0</td>
<td>−8.3</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>6.7</td>
<td>−7.8</td>
<td>−6.1</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>2.2</td>
<td>−6.7</td>
<td>−7.2</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.7</td>
<td>−4.0</td>
<td>−2.0</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.3</td>
<td>−5.0</td>
<td>+0.5</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.08</td>
<td>−1.0</td>
<td>+1.0</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.03</td>
<td>−3.0</td>
<td>−0.5</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.0</td>
<td>−1.0</td>
<td>−2.0</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calculated only for animals that died.

1 $P < .05$, Fisher’s exact test (2-tailed).

2 Paired t test significant at $P = .05$.

When treatment was begun on days 1–3. Toxicity was also seen with a 6.7-mg/kg dose when treatment was begun on day 3 (table 3), which required a shorter treatment schedule. Because substantial infection of the liver occurred by this time, the lack of protection may have been due to altered compound metabolism or pharmacokinetics caused by reduced drug elimination, leading to toxic levels. We did not test lower doses of compound, so we do not know if this was the maximum protection possible.

When treatment was initiated on day 3, higher doses of drug resulted in a significant increase in mean time to death. None of the animals had signs of disease at the end of the treatment period; however, they all died 5–6 days after cessation of treatment. Two treated moribund animals were killed 5 days after the end of treatment; both had virus titers in the liver that were 2 logs lower than those expected of terminal animals, suggesting that viral replication initially may have been partially controlled but resumed after treatment was discontinued.

Treated survivors were rechallenged with 100 pfu (3000 LD_{50}) of virus and, once again, all animals were protected, demonstrating that they were infected.

**Discussion**

The task of developing filovirus chemotherapy is complicated by the constraints imposed in conducting research under BLS-4 conditions, the limited information available on the mechanism of viral replication, and the lack of closely related viruses of lower pathogenicity that could serve as surrogates for drug studies. The recognition that filoviruses share similar gene organization with paramyxoviruses provided a rationale for selecting compounds for initial testing. Inhibition of EBO virus replication by several compounds known to inhibit SAH hydrolase led us to investigate a series of known inhibitors, nine of which we report here. All SAH hydrolase inhibitors tested inhibited viral replication, and where their inhibition
Table 3. Effect of treatment with carbocyclic 3-deazaadenosine (Ca-c3Ado) on survival of 6-week-old BALB/C mice infected with 30 LD50 of Ebola virus.

<table>
<thead>
<tr>
<th>Day treatment begun</th>
<th>Dose (mg/kg) every 8 h</th>
<th>Treatment days</th>
<th>No. survived/total % survival</th>
<th>Mean time to death* (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0–8</td>
<td>1/20 5 6.7 (0.9)</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>0–8</td>
<td>10/10 100</td>
<td>² 100 Ð 12.3 (5.7)</td>
<td></td>
</tr>
<tr>
<td>6.7</td>
<td>1–9</td>
<td>10/10 100</td>
<td>² 100 Ð 15.0 (3.4)</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>1–7</td>
<td>7/10 70</td>
<td>² 100 Ð 15.0 (2.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2–10</td>
<td>5/10 50</td>
<td>² 100 Ð 15.0 (3.4)</td>
<td></td>
</tr>
<tr>
<td>6.7</td>
<td>2–10</td>
<td>9/10 90</td>
<td>² 100 Ð 15.0 (3.4)</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>2–7</td>
<td>0/10 0</td>
<td>² 100 Ð 9.2 (0.8)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3–11</td>
<td>4/10 40</td>
<td>² 100 Ð 9.2 (0.8)</td>
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</tr>
<tr>
<td>6.7</td>
<td>3–11</td>
<td>1/10 10</td>
<td>² 100 Ð 9.2 (0.8)</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>3–8</td>
<td>0/10 0</td>
<td>² 100 Ð 9.2 (0.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated only for animals that died.
² P < .05, Fisher’s exact test (2-tailed); Kaplan-Meier survival analysis log-rank test (Mantel-Cox) and Breslow (generalized Wilcoxon) test statistics were significant at P < .05.
³ Paired t test significant at P = .05.

constant was known [19], it correlated with the EBO IC50 (data not shown). The specificity of inhibition was shown by the lack of antiviral activity of guanosine dialdehyde (not an inhibitor of SAH hydrolase) compared with that of adenosine dialdehyde. SAH hydrolase has been recognized as an important target for antiviral drug development for some time [8].

Antiviral activity of Ca-c3Ado in vitro and in vivo was in the range reported for other viruses, including respiratory syncytial virus and parainfluenza virus [7, 20]. Initial evaluation with dosing either once or twice per day demonstrated moderate antiviral activity [20]. Studies with c3-Npc A in a SCID mouse model showed that while dosing every 12 h only reduced virus titers by 1–2 logs, dosing every 8 h reduced the titer by >6 logs (unpublished results). Ca-c3Ado was initially evaluated in a SCID mouse model, in which it significantly slowed the rate of viral replication and increased the mean time to death (unpublished observations); however, this model was far from ideal for drug evaluation.

Our efforts to adapt EBO virus to produce lethal disease in immunocompetent adult mice by serial passage is published elsewhere in this supplement [10]. Adult BALB/c mice infected by ip inoculation with 30 LD50 of mouse-adapted EBO virus become ill within 3–4 days and uniformly die on days 5–7. The virus infects cells of the mononuclear phagocytic system, hepatocytes, and endothelial cells. Pathologic changes in the liver and spleen resembled those seen in EBO-Z infection of humans and nonhuman primates. Viral replication is first detectable on day 2 and leads to a rapid rise of virus titers in serum and tissues, reaching near maximum titers by day 4. This pathology resembles that observed in primates dying of EBO infection [10]. While this is clearly a severe model of viral infection, it appears appropriate as a model for the human disease.

Our initial concern was that the model would present a formidable challenge for antiviral intervention. The design of initial prophylactic studies had the benefit of appropriate pharmacokinetic studies that established the requirement for dosing at least every 8 h. The treatment duration was empirically selected as 1.5 times the expected mean time to death, and this parameter had not been evaluated to determine if this is optimal. Studies to determine the therapeutic window for intervention showed that treatment could be initiated quite late in the disease process on day 3, when 107–108 pfu/g virus was present in organs. These animals were symptomatic when treatment was initiated and recovered slowly over the next 2 weeks.

Later times of initiation of therapy have not yet been investigated because increasing drug toxicity, observed when treatment was initiated on day 2 or 3, suggested that either sufficient organ damage occurred to limit drug dosage due to increased sensitivity or that drug pharmacokinetics had been altered because the rate of drug elimination had changed. Elimination of Ca-c3Ado from tissues of healthy mice is rapid and correlates with the appearance of a metabolite in serum that has yet to be identified [17]. Significant liver necrosis was seen by days 2 to 3. The liver is involved in the clearance of many nucleoside analogues. Additional studies of drug levels in mice 2–4 days after infection will be required to design studies to determine how late in the infection process treatment can be initiated. Nevertheless, Ca-c3Ado is the first compound reported to cure animals from this otherwise lethal infection.

SAH hydrolase is a highly conserved enzyme among species, and the virus IC50 of SAH hydrolase inhibitors does not differ significantly among cells derived from human, nonhuman primate, and murine tissues. Optimization of conditions for Ca-c3Ado delivery dramatically improved antiviral activity, compared with published results of activity against other viruses.
treated under less than optimal conditions. Two members of this class of nucleoside analogues had similar short serum half-lives, which suggests the need to reinvestigate the in vivo activity of other members of this class. The therapeutic window found for Ca-c^3Ado in this EBO mouse model supports the potential of this compound for therapy of filovirus infection, as does the activity in nonhuman primates who were infected with respiratory syncytial virus and dosed every 12 h (Soike K, personnel communication). The availability of an EBO virus nonhuman primate model [3] will facilitate further evaluation of this compound.

Acknowledgments

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References