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Inhibition of Feline Immunodeficiency Virus (FIV) Replication by DNA Binding Polyamides

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Abstract—Two DNA minor-groove binding polyamides **1** and **2** were designed and synthesized and evaluated for inhibition of FIV-34TF10 replication. Both **1** and **2** decreased the replication of FIV-34TF10 by 75% by acting at the level of the virus but outside of the LTR or env region.

One of the main reasons lentivirus infections are so difficult to treat is the virus ability to adapt to various conditions through rapid mutation. This ability has allowed Human Immunodeficiency Virus (HIV) to develop drug-resistant mutants in patients that have been under treatment for six months or longer.[1]

Although the combination therapies have shown a great deal of promise in treating HIV-1-infected people, it is clear that such therapies are not a cure.[2,3] Therapies have not eliminated the virus from the body[3] and given the propensity of this virus to mutate and adapt, viral resistance has developed in patients who have received the combination therapies.[2] It is because of all of these reasons that continuing investigations into new therapeutic approaches are needed.

A suitable animal model would be extremely useful in designing experimental approaches to answer basic questions pertaining to pathogenesis of lentiviruses, screen promising new antivirals, assessing the suitability of future chemotherapeutic targets, and compare the different drugs and chemotherapeutic approaches in their ability to alter the course of lentivirus induced diseases. Feline immunodeficiency virus (FIV), isolated virus of domestic cats,[4] has been shown to be a useful model for the studying of inhibition, drug resistance and vaccines.[5] Although FIV is not known to infect people, it is closely related to HIV. Both FIV and HIV are retroviruses in the lentivirus subfamily and cause a fetal immunodeficiency syndrome in their respective hosts (cat and humans, respectively).[4,6]

One of the most important strategies in the pharmacology concerning HIV is focused on the development of new drugs or on the modification of existing drugs. Among the new and promising antiviral agents are compounds that interact reversibly with nucleic acids, and in particular to the ones related to the polyamide prototypes netropsin and distamycin.[7] Sulfonated and phosphonated distamycin derivatives capable of suppressing HIV-1 replication and some bis-polyamides have shown HIV-1 IN (integrase) inhibition in nanomolar concentrations are of potential therapeutic importance.[8] Recently, oligo-1,3-thiazolecarboxamide analogues of distamycin have shown that they are more powerful and versatile inhibitors of reverse transcriptase dependent polymerization than the natural product.[9] Since FIV is a useful model in the search for drugs for HIV, we synthesized two polyamides, one with a tripyrrolocarboxamide with a carboxylic benzene ring as the amino terminus of the polyamide **1** and one with an aminothiazole diimidazolecarboxamido polyamide **2** having an amino group as the leading end on the thiazole heterocycle, to test on FIV models (Fig. 1).

Chemistry

Polyamide **1** was synthesized using our reported procedure[8b] while **2** was synthesized by the catalytic hydro-

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genation of nitrodimidazole dicarboxamide **I**[10] using Pd/C (10%, 250 mg) (500 mg, 1.32 mmol) in DMF/MeOH (1:1, v/v, 10 mL) which afforded an unstable amine that was dried, after filtering off the catalyst in order to remove the traces of MeOH, and redissolved in dry DMF. To the latter solution was added 2-amino-4-methylthiazole-5-carboxylic acid[10] (210 mg, 1.32 mmol) dissolved in dry DMF (5 mL), followed by the addition of 1-hydroxybenzotriazole (250 mg, 1.85 mmol) and 1,3-dicyclohexylcarbodiimide (380 mg, 1.85 mmol) (Scheme 1). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was filtered and purified by flash chromatography (2:8:0.2, MeOH/CH_2Cl_2/NH_4OH, v/v/v) afforded **2** in pure form (250 mg, 30% yield) as yellow crystals.[11]

Biological Evaluation

Cell line

For these studies we used the fetal glial cell line G355-5, which is chronically infected with 34TF10 or the PPR-34TF10env chimeric virus and was described as an infectious molecular clone of FIV. G355-5 cells were maintained in Dulbecco's modification of minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, sodium pyruvate (0.11 mg/mL), and gentamicin sulfate (50 μg/mL).

Viruses

The infectious molecular clone of FIV-34TF10 was used as a virus for this study and was prepared as previously described.[6,12,13] FIV-34TF10 was established from a tissue culture adapted strain of the Petaluma isolate of FIV and replicates efficiently in the astrocyte G355-5 cell line.[13] The FIV-PPR clone was established from the San Diego strain of FIV and has a host cell range of macrophages and lymphocytes but does not replicate efficiently in G355-5 cells.[11] To allow for a comparision of the effects of the polyamides on two viral strains in G355-5 cells, a chimeric virus, PPR-34TF10env, was used in this study. PPR-34TF10env has approximately 66% of the 34TF10's 3′ env region and efficiently replicates in the G355-5 cells.[14]

Reverse transcriptase (RT) assay

Virus containing supernatants were centrifuged at 230,000*g* for 45 min at 22 °C. The tubes were drained and swabbed to remove any residual medium, then the viral pellets were resuspended thoroughly in 100 μL reaction mixture (20 mM DTT, 40 mM Tris, 360 nM NaCl, 2% NP40). The samples were frozen/thawed twice in dry ice/ethanol bath to disrupt virions, then 25 μL were mixed with an equal volume of buffer containing 40 mM Tris–HCl, pH 8.1, 60 mM NaCL, 0.02 U poly rA dT (Pharmacia-Biotech), 0.625 μCi 3H-dTTP (80 Ci/mmol, NEM, Boston, MA, USA), and either 6 mM $MgCl_2$ or 1.2 mM $MnCl_2$ (negative control). After incubation for 1 h and 30 min at 27 °C, the samples (in quadruplicate) were spotted on Whattman DE81 filters then washed, first with 0.1 M sodium pyrophosphate and then with 0.3 M ammonium formate. The filters were then washed with 95% ethanol, dried under a heat lamp and examined for radioactivity by liquid scintillation counting (Packard). The protein content was measured with Bradford assay[15] in 100 μL of the remaining lysate and expressed in μg/mL. The results were expressed as cpm of RT activity/μg of protein.

Chloramphenicol acetyl-transferase (CAT) assay

Transient cotransfections were carried out in 6- and 12-well plates using LTR34TF10-dependent CAT expression on plasmid (pLTR34TF10-CAT). The LTR34TF10-dependent plasmid (pLTR34TF10-CAT) was made by

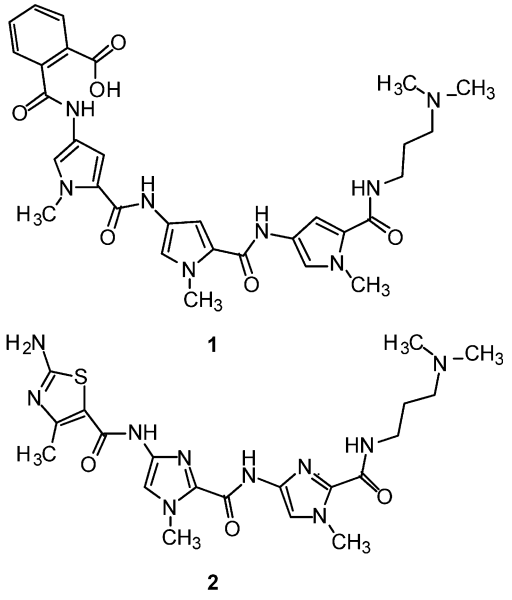
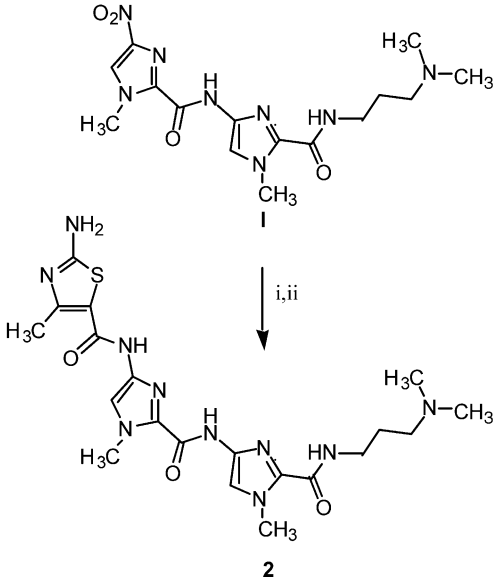


Figure 1.



Scheme 1. Reagents and conditions: (i) Pd/C, DMF/MeOH (1:1), H_2 (gas), 2 h; (ii) 2-amino-4-methylthiazole-5-carboxylic acid.

cloning LTR34TF10 of FIV-34TF10 into pCAT3-Basic (Promega) using the XhoI and SalI sites allowing the LTR to drive the expression of the CAT gene. A lipofectin based transfection protocol DOTAP (Boehringer Mannheim) was used with 10 μg of the plasmid DNA for each sample. OptiMEM (Life Technologies) was the media used during the transfections in 1 mL volumes, then removed after 6-h incubation in normal DMEM with 10% FBS. The cells were harvested with a cell scraper, placed into ice cold PBS and then resuspended in 0.25 M Tris pH 7.5. The cells were disrupted with two freeze/thaw cycles in dry ice/EtOH bath at 37 °C. The cellular lysates were incubated at 37 °C for 1 h with ^{14}C-chloramphenicol (Amersham CUA435) as the reporter and butyryl-CoA as the substrate. Samples were run on TLC plates (Baker) and exposed to Bio Max (Kodak) single-sided emulsion film for clarity and densities were measured using the Investigator Series Video Capture Device (Fotodyne). The data were then compiled and processed using the GelPro software (Media Cybernetics) and are represented as percentage of acetylation.

Statistical analysis

Depending upon the outcome of Barlett's test for homogeneity of variances data were analyzed by either one-way analysis of variance (ANOVA) or Kruskal–Wallis nonparametric ANOVA test. If the ANOVA analysis demonstrated significant differences between groups, then the various treatment groups were compared with the control groups, using either Dunn's multiple comparisons test (for nonparametric data) or Bonferroni multiple comparisons test (for parametric data). Data were considered significantly different from the control when $p < 0.05$.

Results and Discussion

The results of this study indicate that both **1** and **2** significantly decreased the replication of FIV-34TF10 (Fig. 2a) However, neither **1** and **2** significantly affected the replication of FIV-PPR-34TF10env, indicating a high-selective effect (Fig. 2b).

The polyamide **1** effects on replication of FIV-34TF10 were statistically significant at the 0.1, 10, 100, and 1000 nM concentrations. Polyamides **1** and **2** both decreased the replication of FIV-34TF10 by 75% at a concentration of 0.1 nM. To examine the possibility that **1** and **2** affected FIV-34TF10 replication by acting at the LTR level of the virus, the LTR of FIV-34TF10 was cloned into a CAT expression system so that the LTR of FIV-34TF10 was driving the CAT gene expression. It was found that neither **1** nor **2** significantly affected CAT gene expression (Fig. 3).

Thus it was concluded that **1** and **2** affected the replication of FIV-34TF10 by other means than binding to the LTR region of the virus. It can also be concluded that the env region of the virus was not the site of action for the polyamides for both FIV-34TF10 and FIV-PPR-34TF10env contained the same env sequences, and the replication of FIV-34TF10 was significantly decreased, while the replication of FIV-PPR-PPR-34TF10env was not. Since both viruses were grown in the same cells a direct effect of the polyamides on the cells is unlikely.

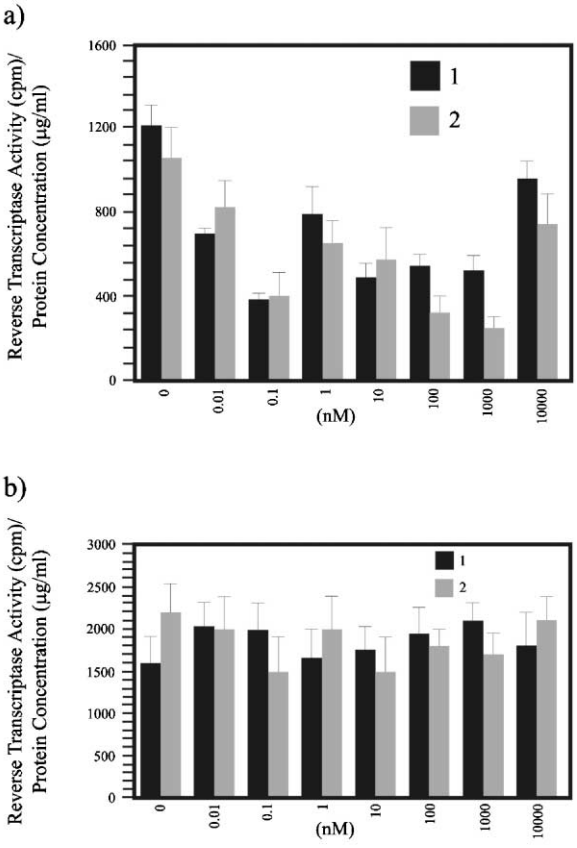


Figure 2. Effects of polyamides **1** and **2** on the replication of FIV 34TF10 (a) or FIV PPR34TF10env (b) in chronically infected feline glial cell lines G355-5 FIV chronically infected astrocytes cell line G355-5 were seeded at 50,000 cells per well at day 0. Then, 1 day later, the cells were incubated at the indicated polyamides concentrations for 2 days. Supernatants were then harvested and a reverse transcriptase assay performed and protein concentration determined as described in text. Results are expressed as the ratio of mean cpm ± standard error of the mean of RT activity per μg/mL of protein concentration. * = $p < 0.05$.

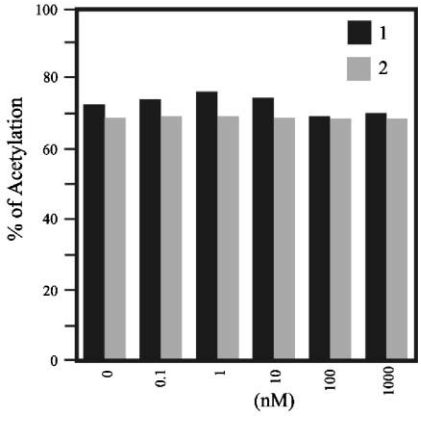


Figure 3. Effects of polyamides **1** and **2** on LTR34TF10-CAT reporter. Note the absence of effects of polyamides **1** and **2** on the pLTR34TF10-CAT at the doses 0.1–1000 nM. Results are expressed as the percentage of acetylation.

Thus the results of this study can be interperated that **1** and **2** decreased the replication of FIV-34TF10 by acting at the level of the virus but outside of the LTR or env region. Further studies to identify the precise mode of action of these two polyamides on FIV-34TF10 replication are in progress and will be reported in due course.

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