4'-Modified NRTIs’ Potent Anti-HIV Activity Stems from Strong RT Active Site Binding

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Abstract

4-Ethynyl-2-fluoro-2-deoxyadenosine (EFdA or MK-8591) (Figure 1), a novel nucleoside reverse transcriptase inhibitor (NRTIs) under clinical trials (Figure 2), is one of the most potent and long-acting anti-HIV-1 agents. EFdA and its derivatives, unlike other conventional NRTIs, possess a modified 4'-moiety while retaining the 3'-OH group in the ribose, and potently inhibit HIV-1 strains resistant to currently available NRTIs. In the present study, we found that EFdA and derivatives with 4'-ethynyl or 4'-cyano moiety retain activity against HIV-1 variants and a multi-NRTI-resistant HIV-1 (HIV-1ΔM184V), but not NRTIs with other moieties examined (e.g., 4'-methyl). Structural study based on a newly defined crystal structure of HIV-RT complexed with EFdA-TP revealed that EFdA and 4'-ethynyl/cyano-NRTIs (but not other 4'-modified NRTIs) examined, had little vDNA interactions with RT’s residues such as F160, and the binding persisted even in the presence of the broadly resistance-endowing V184, thus potently exerting activity against drug-resistant HIV-1s.

Introduction

EFdA (Figure 1), which our group developed in collaboration with Yamasa Corporation and others (Nakata et al. AACC 2007; Kawamoto, et al. Int J Biochem Cell Biol 2008; Hattori et al. AAC 2009; Murphy- Cord et al. AAC 2010; Maeda et al. Antiviral Therapy 2013; Yamashita et al. AAC 2014), represents a novel anti-HIV-1 nucleoside reverse transcriptase inhibitor (NRTIs) currently under clinical trials in the United States. EFdA exerts highly potent activity against a wide range of drug-resistant HIV-1s and the emergence of EFdA-resistance HIV-1s significantly delays compared to other NRTIs such as FTC, TDF, and TDF (Kawamoto, et al. Int J Biochem Cell Biol 2008; Maeda et al., AvT 2014). EFdA possesses highly favorable PK profiles; Grobler et al. have reported that 10 mg single dose administration achieved clinically effective concentrations for at least 7 days, and long-acting parental formulations of EFdA achieved continuous drug release giving high plasma levels for 6 months or longer (Grobler et al. OCHO 2016). These results strongly suggest the EFdA has at least a OW (once weekly) oral dosing capability and presumably a further extended dosing regimen capability with long-acting parenteral formulations.

In the present study, we newly synthesized various 4'-modified NRTIs, all structurally related to EFdA, tested their antiviral activity against a variety of NRTI-resistant HIV-1 strains including a highly multi-drug resistant HIV-1 (HIV-1ΔM184V), found that EFdA and certain 4'-modified NRTIs maintain their potential activity against HIV-RT carrying drug resistant mutation(s), and studied the mechanism based on a recently defined crystal structure of EFdA-TP complexed with RT (Li, Saile, Mitsuya, Sarafianos et al. PNAS 2016).

Results 1

Figure 1. Structure of 4'-Ethynyl-2-fluoro-2-deoxyadenosine (EFdA/MK-8591)

Figure 2. TDF 300 mg QD (once daily)

Figure 3. Structure of 4'-modified NRTIs examined in this study

Activity of 4'-modified NRTIs against wild type HIV-1. We designed and synthesized various 4'-modified NRTIs (4'-NRTIs; Figure 3) and determined their activity against wild type HIV-1 (HIV-1WT). When examined with the MTT assay using MT-4 cells, all the 4'-NRTIs examined were active against HIV-1. Among them, seven NRTIs including EFdA and CADA (4'-C-y-cyano-2-amino-2'-deoxyadenosine) (Takamatsu et al. 2016) had highly potent activity with IC50 values less than 10 nM (Table 1).

Activity of 4'-NRTIs against NRTI-resistant HIV-1. We previously selected an HIV-1 variant resistant against EFdA (EFdAΔM184V), which carries M184L+T215Y+K70R+L74I substitutions (Maeda et al. AvT 2014). We then generated multiple HIV-1 clones containing a part of such mutations in HIV-1 (ΔM184V, ΔT215F, ΔK219Q substitutions). Figure 4A). These results strongly suggest the 4'-NRTIs examined in the present study are divided into two different groups: (i) an HIV-1ΔM184V-active group with 4'-ethynyl or 4'-cyano such as EFdA and CADA and (ii) an HIV-1ΔM184V-inactive group with other 4'-moieties examined such as SK13-204 and SK13-078 (Figure 4B). Interestingly, the five 4'-NRTIs also maintained activity all against HIV-1ΔM184V (Table 1).

Thus, the 4'-NRTIs examined in the present study are divided into two different groups: (i) an HIV-1ΔM184V-active group with 4'-ethynyl or 4'-cyano such as EFdA and CADA and (ii) an HIV-1ΔM184V-inactive group with other 4'-moieties examined such as SK13-204 and SK13-078 (Figure 4). Moreover, we found that the interactions of EFdA with the 3 AA residues of HIV-RT active site, Tyr115 (C), Phe160 (E), and Asp185 (E) are maintained in all the available 4'-modified NRTIs. On the other hand, V184 of HIV-RT interacts with all 4'-modified NRTIs, as well as with wild-type HIV-1 RT (RTWT; Figure 5A), and 4'-ethynyl or 4'-cyano were all active with IC50 ranging from 1.8 to 5.9 nM.

Figure 4. Interaction of EFdA-TP with HIV-1WT (A) and wild HIV-1ΔM184V (B). The topographical plot of the HIV-1WT active site with EFdA-TP is shown. EFdA group of EFdA group has gray with interactions with several residues such as HIV-1WT YMS03072, YMS03076, and CADA in the HIV-RT active site (Figure 5).

Figure 5. Structures of 4'-modified NRTIs examined in this study

Table 1. Anti-HIV activity and 4'-structure of tested NRTIs

Table 3. Results 3

These data strongly suggest that the interactions of EFdA and its derivatives with the 3 AA residues contribute to the potent activity of EFdA against all the mutated HIV-1 variants examined in the present study. It is also thought that the interactions of EFdA with the three AA residues are relevant to the significant delay of HIV-1’s acquisition of EFdA resistance.

Conclusions

The present data on EFdA and its derivatives containing 4'-ethynyl or 4'-cyano may also help design further new NRTIs that strongly bind to an active site residue(s) critical for HIV-RT functionality (e.g. F160).