2′-Deoxy-4′-C-ethynyl-2-fluoroadenosine: A nucleoside reverse transcriptase inhibitor with highly potent activity against all HIV-1 strains, favorable toxic profiles and stability in plasma

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ABSTRACT

A working hypothesis to solve the critical problems of existing HAART was proposed. The study based on the hypothesis proved the validity of the hypothesis and resulted in the development of 2′-deoxy-4′-C-ethynyl-2-fluoroadenosine (4′Ed2FA), a nucleoside reverse transcriptase inhibitor (NRTI) with highly potent activity against all HIV-1 strains, very favourable toxic profiles, and stability in plasma.

INTRODUCTION

HAART has dramatically improved the q, o. l. and prognosis of patients infected with HIV-1. However, the existing HAART has critical problems. They are 1) emergence of drug-resistant HIV variants, 2) requirement of frequent and large doses of drugs, and 3) side effects of drugs.

A working hypothesis to solve the problems was proposed based on the fundamentals of both organic chemistry and biochemistry, and past findings of relationship between biological activity and structure of nucleoside derivatives. The hypothesis is comprised of the following three ways.

1) The way to prevent emergence of drug-resistant HIV

All clinical NRTIs belong to the family of 2′,3′-dideoxy-nucleoside (ddN). The ddN structure has been assumed essential for nucleoside derivative to be anti-HIV active, i.e. to be the chain terminator of proviral DNA biosynthesis. However, HIV variants resistant to all these clinical NRTIs emerged. Resistance to these ddNs means that HIV can acquire the ability to discriminate between ddN and physiologic 2′-dideoxy-nucleoside (dN) and does not accept ddN into the active center of its reverse transcriptase (RT) and/or selectively cut off the incorporated ddN from its proviral DNA terminus. Thus, resistance is the discrimination by HIV. Therefore, the nucleoside (N) that could prevent the emergence of drug-resistant HIV variants must satisfy the following conditions.

(1) To prevent discrimination by HIV, N must have the structure very much like dN. Therefore, N must have 3′-OH.

(2) In spite of having 3′-OH, N must be the chain terminator of proviral DNA biosynthesis:

2′-deoxy-4′-C-substituted nucleoside (4′SdN) was designed as the nucleoside that can satisfy these conditions on the basis of the following hypothesis:

(a) It will be difficult for HIV to discriminate between 4′SdN and dN because 4′SdN has all the functional groups of dN.

(b) The neopentyl-type secondary 3′-OH of 4′SdN would be too unreactive to be used for elongation of proviral DNA biosynthesis. Thus, 4′SdN could be the chain-terminator of proviral DNA biosynthesis.

2) The way to decrease the toxicity of nucleosides

In 1960s and 1970s, organic chemists synthesized nucleoside derivatives modified at two or more than two positions of physiologic nucleosides expecting to get nucleoside derivatives with excellent biological activity. However, none of them showed remarkable biological activity. These results suggested that the intracellular important enzymes do not recognize these modified nucleosides as their substrates. Therefore, the toxicity of 4′SdN could be decreased by additional modification.

3) The way to provide nucleosides with stability to both enzymatic and acidic glycolysis

The lone pair of the ring oxygen plays an important role in both enzymatic and acidic glycolysis of nucleosides by participating to form an oxocarbonium ion. The steric hindrance between the 4′-substituent and 3′-OH of 4′SdN changes the ring conformation into 3′-endo (N-type). It will be difficult for the lone pair of the ring oxygen of 4′SdN with 3′-endo conformation to form oxocarbonium ion because the three bonds, C4-O-C1-C2, can not be co-planar easily. Thus, the introduction of a substituent at the 4′-position of nucleosides provide them with stability to both enzymatic and acidic glycolysis.
RESULTS AND DISCUSSION

Studies based on the hypothesis have proved the validity of the hypothesis. Optimization of the 4’-substituents, bases, and their combinations has resulted in the development of 2’-deoxy-4’-C-ethynyl-2-fluoroadenosine (4’Ed2FA), a nucleoside modified at two positions (4’ and 2) of physiologic deoxyadenosine, which is highly active against all existing HIV strains has low toxicity, and is stable to both enzymatic and acid degradation.

The results of the biological evaluation of 4’Ed2FA

1) Anti-HIV activity:
   EC_{50} (wild type)=0.2 nM; AZT=22 nM, (MDR)=0.14 nM; AZT=15,300 nM, (M184V)=3.1 nM; AZT=10 nM.
2) Toxicity:
   DNA polymerase α: IC_{50}>200 μM,
   DNA polymerase β: IC_{50}>200 μM,
   Human mitochondrial DNA polymerase γ: IC_{50}>10 μM.
   ddA: IC_{50}=0.2 μM
   Mouse Toxicity: No acute toxicity up to 100 mg/kg by both oral and intravenous administration.
3) Stability to enzymatic and acidic degradation:
   Half life time of triphosphate of 4’Ed2FA: T_{1/2}≈18 hr,
   triphosphate of AZT: T_{1/2}=3 hr
   About 50% of the cells were protected against the infection of HIV-1 for 24 hr after removal of extracellular 4’Ed2FA in both MT4 cells and MAGI cells cultured in the presence of 0.1 μM of 4’Ed2FA.
   Completely stable to adenosine deaminase under the conditions where 4’EdA was completely deaminated within 60 min.
   Only a small part (3%) was hydrolyzed under the acidic Conditions of gastric juice (pH 1.06) at 24 °C, while ddA was completely decomposed in 5 min.

SUMMARY

A study on the synthesis and biological evaluation of 4’SdNs was conducted according to a proposed hypothesis based on the fundamentals of both organic chemistry and biochemistry. Old findings proved the validity of the hypothesis and resulted in the development of 4’Ed2FA, which is highly potent against all HIV-1s, is stable to intracellular catabolism and acidic degradation, has a very long intracellular T_{1/2}, does not greatly inhibit DNA polymerase γ and does not have acute mouse toxicity. These results strongly suggest that 4’Ed2FA deserves further study for the development of a highly potent therapeutic agent for HIV infection (AIDS), which may solve the problems of the existing HAART.

In addition, it should be noted that 4’Ed2FA could be the ideal drug for both HIV and HBV infections. Hepatitis B virus (HBV) is a DNA virus, however, it also belongs to the family of retrovirus because it uses reverse transcriptase (RT) when it replicates. It was found that the NRTIs of HIV are also active against HBV, and 3TC has been used for HBV infection. However, HBV resistant to 3TC has emerged. Since 4’Ed2FA is highly active against 3TC-resistant HIV and will prevent the emergence of drug-resistant HIV, it is also expected to be active against drug-resistant HBV. Thus, 4’Ed2FA could be the ideal drug for use against both HIV and HBV.

REFERENCES