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Characterization of the mechanism of action of the ultra-potent HIV inhibitor 4'-Ethynyl-2-Fluoro-2'-Deoxyadenosine

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Retroviruses rely on the enzyme reverse transcriptase (RT) to perform the reverse transcription of its genome from single-stranded RNA into double-stranded DNA, which can then be integrated into the host's genome by the action of the viral integrase enzyme. There are currently sixteen antiretroviral agents used for the treatment of HIV infections. Highly active antiviral therapy (HAART) is based on a combination of at least 3 anti-HIV drugs. It has slowed down the progression of AIDS and decreased mortality. RT is one of the main targets for these antiretroviral drugs. One class of drugs targeting the reverse transcriptase is the nucleoside analogue RT inhibitors (NRTIs). NRTIs compete with natural nucleotides for incorporation in the elongating DNA chain by HIV-1 RT. Once incorporated, they act as chain-terminators because they lackthe 3'OH group which is required for further nucleotide incorporation. Prolonged use of these drugs leads to drug-resistant HIV strains. To overcome drug resistance, novel inhibitors that are active against NRTI-resistant viruses are being developed. NRTIs containing a modification at the 4' position of their sugar moiety have been synthesized by Hiroaki Mitsuya and his colleagues. One of these analogues, 4'ethynyl-2-fluoro-2'-deoxyadenosine (4'-E-2-F dA) was shown to be ultra-potent against wild-type and drug resistant HIV-1. Unlike other nucleoside analogues, 4'-E-2-F dA has a hydroxyl group at the 3' position. The purpose of this project is to understand the mechanism of RT inhibiton by 4'-E-2-F dA. In order to determine its mechanism of action, in vitro primer extension assays as well as gel mobility shift assays were used. Using primer extension assays, we determined that the active form of 4'-E-2-F dA, 4'-E-2-F dA-triphosphate (TP), acts as a chain terminator at physiological concentrations of nucleotides, despite the presence of a 3'OH. We first hypothesized that the presence of 4'-E-2-F dA-monophosphate (MP) at the 3' end of the primer destabilized the RT/DNA complex. The RT/DNA complex is not affected by the presence of 4'-E-2-F dAMP as observed in gel mobility shift assays. We next hypothesised that RT was not able to bind to the next incoming nucleotide to form a ternary complex. Indeed, we found that the presence of 4'-E-2-F dAMP at the 3' end of the primer severly impair the formation of a stable ternary complex. In conclusion, we found that 4'-E-2-F dA inhibits DNA elongation by RT by acting as chain-terminator despite the presence of the 3'OH. In order to do so, 4'-E-2-F dAMP blocks the binding of the next incoming nucleotide. This is a novel mechanism of inhibition that results in the most efficient blocking of HIV activity reported to date for any NRTI. Our findings have generated interest from two pharmaceutical companies that wish to develop it as a next-generation therapeutic for the treatment of HIV infections.