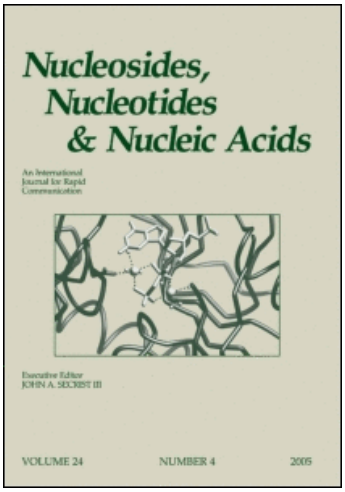

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MECHANISTIC STUDIES TO UNDERSTAND THE INHIBITION OF WILD TYPE AND MUTANT HIV-1 REVERSE TRANSCRIPTASE BY CARBOVIR-TRIPHOSPHATE

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ABSTRACT

Abacavir (1592U89) has recently been approved by the FDA for treatment of HIV infection. Transient kinetic studies were carried out to better understand the interaction of the active metabolite of Abacavir (Carbovir-triphosphate) with wild type and mutant HIV-1 reverse transcriptase. Some of the data is summarized and used as a basis for discussion of inhibition by CBVTP and previously studied nucleoside analogs.

The FDA approved nucleoside analogs d4T (Stavudine) and Abacavir both contain a 2′,3′-unsaturation in the ribose ring which causes the ring to adopt a planar conformation. In addition, Abacavir contains a carbocyclic ring in which a methylene is substituted for the ribose oxygen. Abacavir is a prodrug for Carbovir-triphosphate (CBVTP) (1, 2), which is thought to be responsible for anti-HIV activity by acting as a chain terminator to the elongation of viral transcripts by reverse transcriptase (RT) (3). Prolonged passage of HIV infected cells in the presence of Abacavir leads to the mutation of methionine 184 to valine within the viral encoded RT, and a small decrease in antiviral activity (4).

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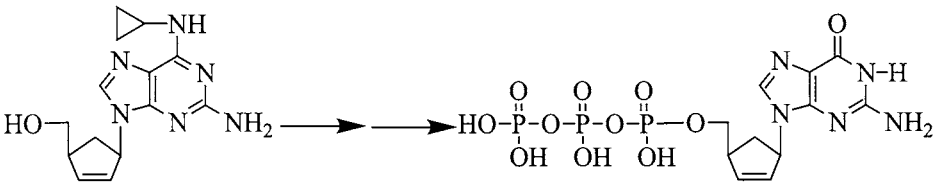

Figure 1. Abacavir is metabolized to the dGTP analog CBVTP.

RESULTS

A transient kinetic approach was used to provide insight at a molecular level for the interaction of CBVTP (Fig. 1) with RT^{WT} and RT^{M184V} and the molecular mechanism of drug resistance. In contrast to steady state methodology, the interaction between the deoxy nucleotide triphosphate (dNTP) and the enzyme active site can be directly studied using transient kinetic methodology (5). By determining the affinity of the enzyme-primer/template complex for dNTP (K_d) and the maximum rate of incorporation (k_{pol}) a selectivity value for wild type or mutant RT bound to DNA/DNA or DNA/RNA could be derived which compares the efficiency of incorporation of the natural nucleotide (dGTP) with the analog (CBVTP).

As illustrated in Table 1, it was found that for both a DNA/DNA and DNA/RNA primer/template, CBVTP was less efficiently incorporated by RT^{M814V} than by RT^{WT}, while there was no difference in the efficiency of incorporation of the natural substrate, dGTP. This differential incorporation of CBVTP by RT^{M184V} reflects that the mutant enzyme is more selective and less likely to incorporate the drug relative to RT^{WT}. With a DNA/DNA primer/template a 7-fold difference in the selectivity between RT^{WT} and RT^{M184V} for dGTP over CBVTP was observed

Table 1. Efficiency and Selectivity Values of RT^{WT} and RT^{M184V} for dGTP over CBVTP

Primer/template	RT	Nucleotides	Efficiency ($\mu M^{-1}s^{-1}$)[a]	Selectivity[b]
DNA/DNA	WT	dGTP	1.7 ± 0.3	34
		CBVTP	0.05 ± 0.01	
	M184V	dGTP	2.2 ± 0.3	240
		CBVTP	0.009 ± 0.001	
DNA/RNA	WT	dGTP	3.1 ± 0.6	7.4
		CBVTP	0.42 ± 0.06	
	M184V	dGTP	2.9 ± 0.8	170
		CBVTP	0.017 ± 0.004	

[a]Efficiency $= k_{pol}/K_d$.
[b]Selectivity $= (k_{pol}/K_d)_{dGTP}/(k_{pol}/K_d)_{CBVTP}$, or $\text{efficiency}_{dGTP}/\text{efficiency}_{CBVTP}$.
[c]Data was generated by doing experiments with a 30-mer DNA primer and a 45-mer DNA or RNA template where the next correct nucleotide to be incorporated was dGTP, similar to techniques used in a previously published report (Feng and Anderson 1999).

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(selectivity values of 34-fold and 240-fold respectively). The difference in selectivity between RT^{WT} and RT^{M184V} was found to be larger during incorporation into a DNA/RNA primer/template. RT^{M184V} was 20-fold less likely to incorporate CBVTP than RT^{WT} (selectivity of RT^{WT} for dGTP over CBVTP equal to 7.4-fold and for RT^{M184V} 170-fold, efficiency and selectivity values summarized in Table 1).

DISCUSSION

Previous transient kinetic studies have shown that d4TTP is as good a substrate as dTTP with HIV-1 RT (6). CBVTP's high relative efficiencies of incorporation furthers the evidence that a planar ring conformation in nucleotides is tolerated by polymerases and that alternate substrates that have this type of structure are incorporated favorably (7). Unlike d4TTP, CBVTP is not incorporated as efficiently as its corresponding natural nucleotide (dGTP) despite its predicted planar ring conformation. This may indicate an important role for the oxygen present in the ribose ring of d4TTP, which the carbocyclic ring of CBVTP lacks.

Combination therapy of 3TC (Lamivudine) and AZT (Zidovudine) has proven effective because the M184V mutation selected for by 3TC prevents mutations necessary for AZT resistance (8). Past studies have shown that 3TCTP is incorporated as much as 140-fold less efficiently by RT^{M184V} than by RT^{WT} (9). This value can be compared with CBVTP where the largest difference is only 20-fold. The ability of Abacavir to select for the M184V mutation and the result that CBVTP only shows a small decrease in incorporation *in vitro* may indicate that it has an advantage over 3TC in combination with AZT.

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