NRTIs tenofovir, TAF, TDF, and FTC are inactive against SARS-CoV-2

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Abstract

Purpose

The urgent response to COVID-19 pandemic required accelerated evaluation of many approved drugs as potential antiviral agents against the causative pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Using cell-based, biochemical, and structural approaches, we studied the approved nucleoside/tide reverse transcriptase inhibitors (NRTIs) tenofovir (TFV) and emtricitabine (FTC), as well as prodrugs tenofovir alafenamide (TAF), and tenofovir disoproxil fumarate(TDF) for their antiviral effect against SARS-CoV-2

Methods

Three approaches were used to evaluate the selected NRTIs: (1) cell-based antiviral assays in A549-hACE2 cells and primary normal human bronchial epithelial (NHBE) cells. Remdesivir (RDV) was included as a positive control. The levels of active intracellular metabolites TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTC-TP) were measured in parallel by LC/MS; (2) TFV-DP and FTC-TP together with the active metabolite of RDV (RDV-TP) were tested for their incorporation efficiency into RNA by the SARS-CoV-2 RNA-dependent-RNA polymerase (RdRp); (3) structural models of the pre-incorporated active metabolites were generated for the SARS-CoV-2 RdRp and compared to existing HIV-1 reverse transcriptase (RT) x-ray structures.

Results

None of the tested HIV NRTIs showed any significant in vitro anti-SARS-CoV-2 effect at concentrations up to 100-fold higher than the clinically relevant levels despite high levels of their active metabolites being formed in cells. TFV-DP was incorporated 40,000-fold less efficiently than its natural counterpart ATP by SARS-CoV-2 RdRp, while the incorporation of FTC-TP was undetectable. In contrast, RDV-TP was incorporated 3.6-fold more efficiently than ATP. These results are consistent with the structural analysis concluding that the 2'OH group, which is lacking in all the tested NRTIs, is a key component of nucleotide recognition by the SARS-CoV-2 RdRp.

Conclusion

A comprehensive set of in vitro data indicates that TFV, TAF, TDF, and FTC are inactive against SARS-CoV-2. These results are corroborated by the lack of interaction between the respective NRTI-TPs and SARS-CoV-2 RdRp observed both in biochemical assays and in structural modeling analyses. Clinicians and researchers should exercise caution when using these and other NRTIs to treat or prevent COVID-19

Results

Table 1: TAF, TDF, TFV, and FTC are not active against SARS-CoV-2 in cell-based assays

Compounds	Cell-based Antiviral Assay EC_{50} (μM)		Clinical Drug Exposure and Active Metabolite Levels in PBMC	
Nucleoside/tide Analogues	A549-hACE2 Cells NanoLuc reporter[1]	NHBE Firefly Luc reporter[2]	Drug Exposure (μM)[3] [Active metabolite]	Plasma protein binding (%)
Remdesivir (RDV)	0.067 ± 0.020	0.0372 ± 0.0166	C_{max} = 7.3, 3.7** [10.2 μM in PBMC]	88
Tenofovir alafenamide (TAF)	>50	>50	C_{max} 0.4	80
Tenofovir disoproxil fumarate (TDF)	>50	>50	Not detectable	-
Tenofovir (TFV)	>500	Not done	Not detectable	-
Emtricitabine (FTC)	>50	>50	C_{max} 7.9	4

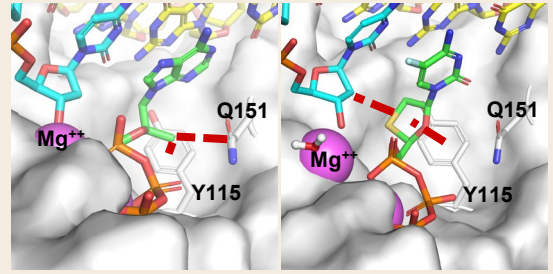
[1] Values are mean ± standard deviation of two independent replicates in A549-hACE2 cells (human alveolar epithelial cell line expressing human ACE2). TAF, TDF, and FTC showed no cytotoxicity up to 50 μM after a 2-day incubation. TFV showed no cytotoxicity up to 500 μM after a 2-day incubation. RDV CC_{50} = >16.7 μM in the 2-day assay.
[2] Values are mean ± standard deviation of 3 independent replicates in NHBE cells (normal human bronchi epithelial cells).
[3] Values represent C_{max} and C_{min} for human exposures EC_{50}, 50% effective concentration; uM, micromolar, ** 200, 100 mg IV 30-min infusion.

Table 2: TAF, TDF, and FTC formed high levels of active metabolites in cells, but these active metabolites are poor substrates of SARS-CoV-2 RdRp

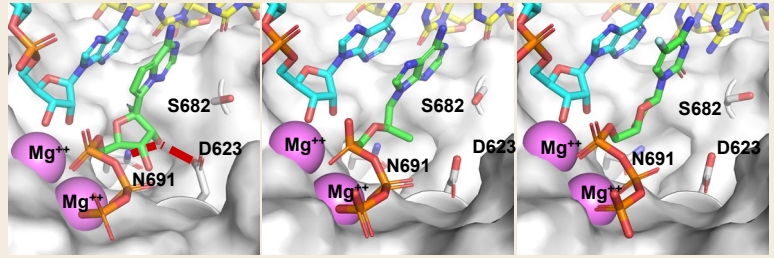
Compounds	Active Metabolites	Biochemical Viral RNA Polymerase Assay[1]	Active Metabolite Formed in Cell Culture (μM)	Extrapolated Active Metabolite under EC_{50} concentration (μM)[2]	Metabolite Level under Clinical Exposure (μM)
Nucleoside /tide Analogues	5'-triphos-phate	Incorporation Efficiency [Relative to natural NTP (fold)]	in A549 at 24-hr	in A549 at 24-hr	Post multiple doses
RDV	RDV-TP	84 [3.6-fold higher than ATP]	21.0 ± 2.2 (with 1 μM RDV)	1.4	10.2 (with RDV 200/100 mg)
TAF	TFV-DP	0.0003 [40,000-fold lower than ATP]	126 ± 50 (with 1 μM TAF)	> 6,300	1 (with TAF 25 mg QD)
TDF			14 ± 2 (with 1 μM TDV)	> 700	0.2 (with TDF 300 mg QD)
TFV			1.7 ± 0.6 (with 10 μM TFV)	NA[4]	NA[4]
FTC	FTC-TP	Too low to be measured	5.8 ± 1.8 (with 1 μM FTC)	> 290	20 (with FTC 200 mg QD)

[1] The 5'-triphosphate forms of each drug was evaluated for the SARS-CoV-2 RNA polymerase-catalyzed incorporation efficiency. The analogs' incorporation efficiency is expressed as the quotient of the Michaelis-Menten parameters Vmax/Km, while comparison of efficiencies is expressed as fold difference from the efficiency of incorporation of ATP (for remdesivir, TAF, and TDF) and CTP (for FTC).
[2] Calculated from the active metabolite levels measured in the A549 cells treated with 1 or 10 μM of drugs and the EC50 values reported in Table 1. A549 cell volume was reported as 1.67 pL by literature.
[3] NA = Not applicable

Figure 1: Residues that govern selectivity for deoxyribose substrates in HIV-RT and ribose substrates in SARS-CoV-2 dictate the efficacy of TFV-DP and FTC-TP



HIV-RT/TFV-DP HIV-RT/FTC-TP



SARS-CoV-2/RDV-TP SARS-CoV-2/TFV-DP SARS-CoV-2/FTC-TP

The active site of HIV-RT creates a hydrophobic environment around the 2' position of the substrate, which selects for deoxyribose substrates over ribose substrates. The hydrophobic surface generated by Y115 and Q151 is ideal for inhibitors such as TFV-DP. The lack of a 2'OH on the primer also creates room for the L-nucleoside inhibitor FTC-TP. In contrast, the active site of SARS-CoV-2 creates a polar environment around the 2' position, which selects for ribose substrates over deoxyribose substrates. RDV-TP is well-recognized by the active site, but TFV-DP and FTC-TP lack the requisite 2'OH to hydrogen bond to residues D623, S682 or N691. FTC-TP also suffers from a clash with the 2'OH of the primer.

References: **1**. Callebaut et al. (2017) PLOS ONE 12(2): p1-11; **2.** Wang et al. (2004) AIDS Research and Human Retroviruses 20(11): p1173; **3**. Jiang et al. (2010) Romanian Journal of Morphology and Embryology 51(4): p663-667. **4**. Gordon C, et al. J. Biochemistry 2020, 295(20):6785-6797. **5**. Xie X, et al. Nature Communications 2020, 11:5214

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