Supplemental Materials

Contributions of Cathepsin A and Carboxylesterase 1 to the hydrolysis of Tenofovir Alafenamide in the Human Liver, and the Effect of CES1 Genetic Variation on Tenofovir Alafenamide Hydrolysis

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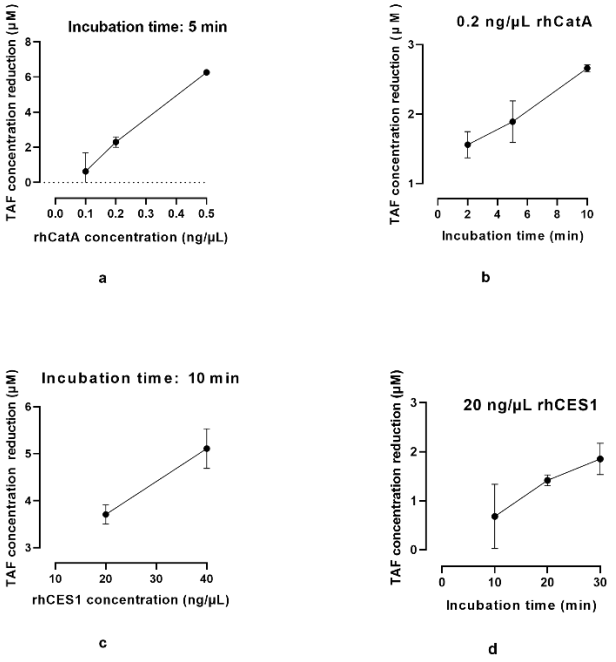
LC-MS/MS assays for TAF and TFV quantification

TAF was quantified based on a previously reported method with some modifications (Avataneo et al., 2020). The LC-MS/MS system consisted of a PE SCIEX API 3000 spectrometer and a Shimadzu UFLC system (Shimadzu, Tokyo, Japan). Chromatographic separation was obtained on a RESTEK Ultra II C18 column (5 µm, 50 × 2.1 mm, Bellefonte, PA) protected by a RESTEK UltraShield UHPLC precolumn filter (0.2 µm frit, Bellefonte, PA) at 45 ^{o}C. The mobile phase flow rate was 0.5 mL/min. The injection volume was 1 µL. The gradient elution was obtained using water containing 0.1% (v/v) formic acid (phase A) and ACN containing 0.1% (v/v) formic acid (phase B) with the following time program: mobile phase B was 2% for the first 0.1 min, increased from 2% to 90% during the time period of 0.1–3 min, maintained at 90% for 0.5 min, then returned to 2% at 3.6 min, and maintained at 2% until the end of the gradient at 5 min. The mass spectrometer was operated in a positive ion mode using turbo electrospray ionization. The following parameters were used for the MS analysis: nebulizer gas: 15 psi; curtain gas: 15 psi; collision gas: 6 psi; ionspray voltage: 5500 V; source temperature: 550 °C; declustering potential: 30 V; focusing potential: 40 V; entrance potential: 10 V; collision energy: 50 V; collision cell exit potential: 15 V. The following transitions were monitored in a multiple reaction monitoring (MRM) mode: TAF, m/z 477.20 > 176.10; RDV, m/z 603.20 > 200.10, scan time: 0.25 second. Quantifications were based on the peak area ratios of TAF to RDV. The regression coefficients of calibration curves were greater than 0.99.

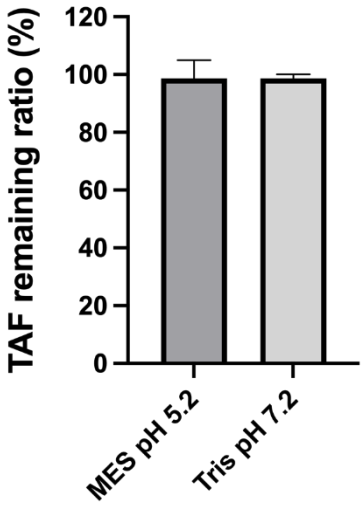
A previously reported method was utilized for TFV quantification with some modifications (King et al., 2006). The LC-MS/MS analysis was performed on an Applied Biosystems API 4000 triple quadrupole/linear ion trap (QTRAP) mass spectrometer (Foster City, CA, USA). Analytes were separated using a Shimadzu HPLC system (Shimadzu, Tokyo, Japan) on a Shimadzu VP-ODS column (5 µm, 150 × 2.0 mm, Shimadzu, Tokyo, Japan). The mobile phase consisted of water with 1% formic acid (phase A) and methanol containing 0.05% formic acid (phase B), and was delivered at a flow rate of 0.2 ml/min. The time program of the gradient elution was as follows: mobile phase B was 2% for the first 3 min, increased from 2% to 88% during the time period of 3–8 min, maintained at 88% for 1 min, then returned to 2% at 9.5 min, and maintained

at 2% until the end of the gradient at 12 min. MS was operated in a positive ion mode using turbo electrospray ionization with the following parameters: curtain gas: 30 psi; ion source gas 1: 40 psi; ion source gas 2: 60 psi; ionspray voltage: 5500 V; source temperature: 500 °C; entrance potential: 10 V; dwell time: 100 ms; collision cell exit potential: 11 V; declustering potential: 60 V; and collision energy: 40 V. The following transitions were monitored in an MRM mode: TFV, m/z 288.2 > 176.3; AFV, m/z 274.6 > 162.4. Quantifications were based on the peak area ratios of TFV to AFV. The regression coefficients of calibration curves were greater than 0.99. The accuracy and precision of both TAF and TFV assays met the requirements in the FDA bioanalytical method validation guidance.

Supplementary Figure 1. Time- and rhCES1 and rhCatA concentration-depenedent TAF hydrolysis. TAF (10 μM) TAF was incubated with (a) rhCatA (0.1, 0.2, 0.5 ng/μL) for 5 min; (b) 0.2 ng/μL rhCatA for 2, 5, and 10 min); (c) rhCES1 (20, 40 ng/μL) for 10 min; d. 20 ng/μL rhCatA for 10, 20, 30 min. Data are shown as the reduced TAF concentration after incubation (mean ± S.D., n = 3).



Supplementary Figure S2. TAF (20 μM) stability following incubation in MES pH 5.2 buffer and Tris pH 7.2 buffer at 37°C for 30 min.



References

Avataneo V, de Nicolò A, Cusato J, Antonucci M, Manca A, Palermiti A, Waitt C, Walimbwa S, Lamorde M, di Perri G, and D'Avolio A (2020) Development and validation of a UHPLC-MS/MS method for quantification of the prodrug remdesivir and its metabolite GS-441524: a tool for clinical pharmacokinetics of SARS-CoV-2/COVID-19 and Ebola virus disease. *Journal of Antimicrobial Chemotherapy* **75:**1772-1777.

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