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Assessment of tenofovir disoproxil fumarate stability in the presence of pancreatic enzymes: implications for prodrug oral absorption and bioavailability

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Introduction: The nucleotide reverse transcriptase inhibitor tenofovir (TFV) is used extensively in first-line antiretroviral therapy. Orally administered TFV has low bioavailability (11%) which is improved (25%) when administered as the prodrug tenofovir disoproxil fumarate (TDF). TDF is degraded to TFV by esterases in intestinal tissue homogenate and blood. However, TDF degradation in luminal fluid, which contains pancreatic enzymes lipase and amylase, has not been fully assessed. This study investigated TDF stability in vitro using pancreatic fluid enzymes and used inhibitors to determine enzyme specificity.

Methods: TDF stability was determined in the presence of a concentration range of pancreatic fluid (0, 1, 3, 10 mg/mL porcine pancreatin, fasted simulated intestinal fluid buffer) and time points (0, 5, 10, 20, 30, 45, 60, 120 min) in a shaking waterbath (20 µg/mL TDF, 100 rpm, 37℃, n ≥ 3). Optimum conditions were then utilised for determining the effects of lipase inhibitor orlistat (100 µg/mL) and amylase inhibitor acarbose (1 mg/mL) on TDF stability (3 mg/mL porcine pancreatin, 20 µg/mL TDF, 100 rpm, 37℃, n = 4). A HPLC method was developed and validated for assessing TDF concentrations and antiretroviral lopinavir was used as an internal standard.

Results: The HPLC method achieved linear standards between 1 and 30 µg/mL TDF. Both intra- and inter-assay TDF quality controls gave acceptable relative standard deviation (<15%) and accuracy (>85%). TDF showed more rapid degradation in higher concentrations of pancreatin ($p<0.05$), resulting in a TDF half-life of 19.4, 10.9 and 5.1 minutes in 1, 3, and 10 mg/mL pancreatin, respectively. Some instability of TDF was detected in the absence of pancreatic enzymes, giving a half-life of 55 minutes. The presence of 100 µg/mL orlistat increased TDF half-life by 40% ($p<0.05$) but the presence of 1 mg/mL acarbose did not significantly affect TDF half-life ($p = 0.81$).

Discussion: Results support the hypothesis that TDF is unstable in pancreatic fluid in vitro, and that this route of degradation may be an additional factor in the determination of TDF oral bioavailability in humans. Enzyme-specific inhibition results support that lipase, and not amylase, is involved in luminal TDF breakdown, suggesting a potential inhibition target for TDF bioavailability boosting strategies in vivo. Additionally, novel formulations may be utilised which protect TDF from pancreatic enzymes, such as delayed or controlled release strategies.