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Differential Pharmacological Impact Of Abacavir Sulphate And Tenofovir On Platelet Aggregation Independent Of HIV Infection

Objectives: HIV infection, when effectively treated with Highly Active Antiretroviral Therapy (HAART), rarely progresses to AIDS and patients live a near normal life expectancy.

Observational and clinical studies have found the nucleoside reverse transcriptase inhibitor (NRTI) abacavir sulphate (ABC), a component of HAART, to be associated with a reversible increased risk myocardial infarction (MI) and increased platelet aggregation in HIV positive patients. It is not clear whether increased cardiovascular risk is driven pharmacologically by ABC or pathophysiologically by HIV and co-morbidities. Since MI is platelet driven, our objective was to assess the impact of the NRTIs ABC and tenofovirdisoproxilfumarate (TDF) or their respective metabolites carbovir triphosphate (CTP) and tenofovir (TVR) on platelet aggregation in vitro and in vivo, independent of HIV infection. Additionally, we aimed to establish whether CTP effected platelet ability to respond to exogenous NO.

Isolated human platelets from healthy consenting donors were stimulated with an approximate EC_{50} concentration of thrombin (0.06 U ml^{-1}) and collagen (0.03 µg ml^{-1}) following 10 minute incubation with an approximate Cmax concentration of CTP or TFV (3 µg ml^{-1}). Additionally, CTP or TVR (50 µm) were incubated with PRP in the absence or presence of the NO-donor S-Nitroso-N-Acetyl-D,L-Penicillamine (SNAP) (5 µM) and stimulated with ADP (3 µM).

The effects of the pro-drugs ABC and TDF (30 µg mL^{-1} administered i.p) were measured on subsequent collagen (50 µg kg^{-1}i.v) induced platelet aggregation *in vivo* in W.T C57 Bl/6 male mice (20-25g) by measuring radiolabelled platelet aggregation in real-time *via* external scintillation probes connected to a spectrometer (1). All animals were anaesthetised using urethane (10 ml kg^{-1} of 25% (w/v) i.p) and procedures were non-recovery. Groups were compared using a Repeated Measures ANOVA, paired or unpaired t-test and all data is presented as mean ± SEM.

TFV significantly inhibited collagen-induced platelet aggregation *in vitro* (24±8.1treated versus 34.6±9.1 vehicle (0.2% DMSO) % aggregation, $P<0.05$, n = 6), however, no effect was detected with CTP (32±5.6 treated versus 34.6±9.1 vehicle (0.2% DMSO) % aggregation, $P>0.05$, n = 5-6). Furthermore, CTP significantly reversed NO-mediated inhibition of ADP-induced platelet aggregation (29.4±6.7 treated versus 15.7±3.1 vehicle (0.3% DMSO) % aggregation, $P<0.05$, n = 7) but no effect was observed for TVR (8.8±4.7 treated versus 6.2±2.7 vehicle (0.3% DMSO) % aggregation, $P>0.05$, n = 5).

In vivo, ABC significantly enhanced collagen (25.8±1.7 vehicle (saline) versus 29.6±3.4 treated AUC for % scintillation counts, $p<0.05$, n = 5) induced platelet aggregation. In contrast, no effect was observed following treatment with TDF (662.1±208.5 treated versus 597.0±73.9 vehicle (saline) AUC for % scintillation counts, $P>0.05$, n = 6).

ABC enhanced platelet aggregation *in vivo* and therefore the increased risk of MI which has been associated with ABC may be platelet-driven. Reported differences between the cardiovascular risk profile of ABC and TDF in patient studies may be due to the retention of endothelial-derived inhibition of platelet activation in the presence of TDF but pharmacological blockade of this cardioprotective mechanism by ABC. Additionally, we report a direct and previously unreported inhibitory pharmacological effect of TFV on platelet function independent of endothelial function which may additionally contribute to the differential morbidity observed in patients treated with ABC or TDF.

Tymvios C et al. (2008) ThrombHaemost, 99(**2**), 435-440.