Cardiovascular Risk Profile of Abacavir and Tenofovir Independent of HIV Infection

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Background

- HIV infection is associated with increased cardiovascular disease (CVD) and the Nucleoside Reverse Transcriptase Inhibitor (NRTI) abacavir sulphate (ABC), a component of antiretroviral therapy (ART), has been linked with increased and reversible risk of myocardial infarction (MI) in observational studies **[1]**.
- MI is a platelet-driven event and ABC is also associated with increased platelet aggregation in people living with HIV (PLWH) **[2]**.
- Platelets are negatively regulated by endogenous endothelial nitric oxide (NO) and impaired NO bioactivity is a driver of CVD.
- It is unclear whether increased risk of MI in PLWH is driven by HIV infection and associated co-morbidities or is a pharmacological side-effect of ART treatment.

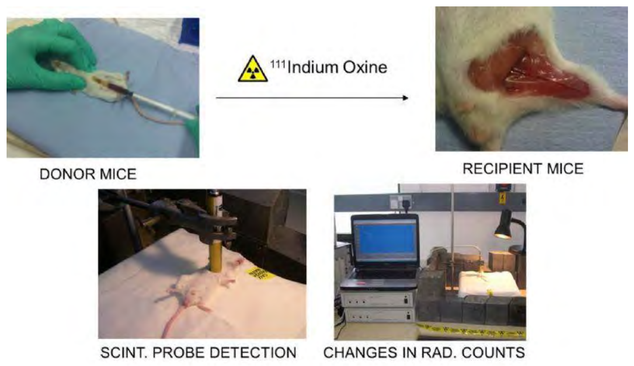
Aims

- To assess the impact the NRTIs ABC and tenofovir disoproxil fumarate (TDF) on cardiovascular risk independent of HIV infection by assessing their effect at clinically relevant concentrations on the function of platelets from healthy volunteers *in vitro* and in a mouse model *in vivo.*
- To establish whether NRTIs affect the regulation of platelets by NO.

Methods

- Platelet aggregation was investigated *in vitro* by measuring changes in light transmission in platelets and platelet-rich plasma from consenting healthy volunteers.
- Platelet aggregation was measured *in vivo* in anaesthetised mice by measuring radiolabelled platelet thromboembolism in the pulmonary vasculature.

Mouse model of platelet thromboembolism



Results

- Tenofovir (TVF), the active metabolite of TDF, significantly inhibited collagen (Fig 1A) but not thrombin (Fig 1B) induced platelet aggregation *in vitro.*
- Carbovir triphosphate (CTP), the active metabolite of ABC, had no effect on agonist-induced platelet aggregation *in vitro* (Fig 1A-B).

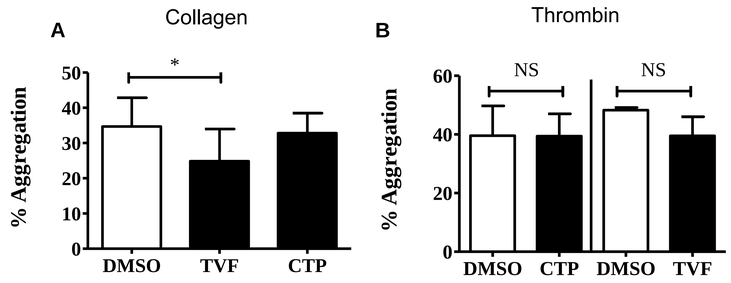


Figure 1. The effect of carbovir triphosphate and tenofovir on thrombin and collagen-induced platelet aggregation *in vitro*.
Isolated human platelets were incubated with carbovir triphosphate (CTP) (3 µg/ml), tenofovir (TVF) (3 µg/ml) or vehicle (DMSO 0.2 %) for 10 mins and then stimulated with EC_{50} concentrations of thrombin (0.06 U/ml) (B) or collagen (0.7 µg/ml) (A) . Platelet aggregation was detected as changes in light transmission and reported as maximum percentage change in light transmission. Data is presented as mean±SEM. *P<0.05, NS=P>0.05 compared to DMSO using a paired t-test. n=6.

- CTP (Fig 2A) but not TVF (Fig 2B) significantly reversed NO-mediated inhibition of ADP-induced platelet aggregation by the NO donor SNAP. The soluble guanylyl cyclase inhibitor ODQ (1H-(1,2,4)oxadiazolo[4,3-a]quinoxalin-1-one) was used as a positive control.

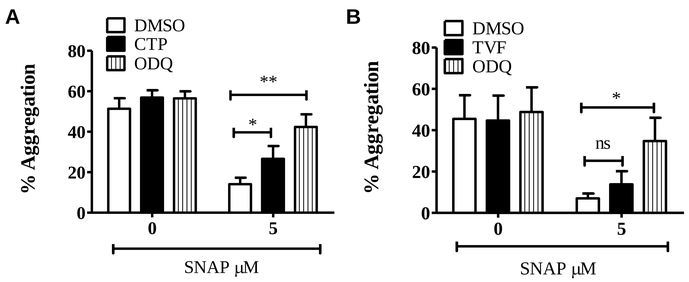


Figure 2. The effect of carbovir triphosphate and tenofovir on NO-mediated inhibition of platelet aggregation *in vitro*.
Platelet rich plasma was incubated with carbovir triphosphate (CTP, 50 µM) (A), tenofovir (TVF, 50 µM) (B) , 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (20 µM) or vehicle (DMSO 0.2 %) for 2 mins prior to the addition of the NO-donor S-Nitroso-N-Acetyl-D,L-Penicillamine (SNAP) (5 µM) for 5 mins and then stimulated with ADP (3 µM). Platelet aggregation was detected as changes in light transmission and reported as maximum percentage change in light transmission. Data is presented as mean±SEM. *P<0.05, **P<0.01, ns=P>0.05 compared to DMSO using a Repeated Measures ANOVA with bonferroni post hoc analysis. n=6-8.

Results

- Acute administration of ABC enhanced collagen-induced platelet aggregation *in vivo* 30 mins after administration whereas TDF had no effect (Fig 3A&C).
- The effect of ABC had dissipated after 4 hours (Fig 3B&D), indicating a reversible effect in line with that reported in human clinical observations

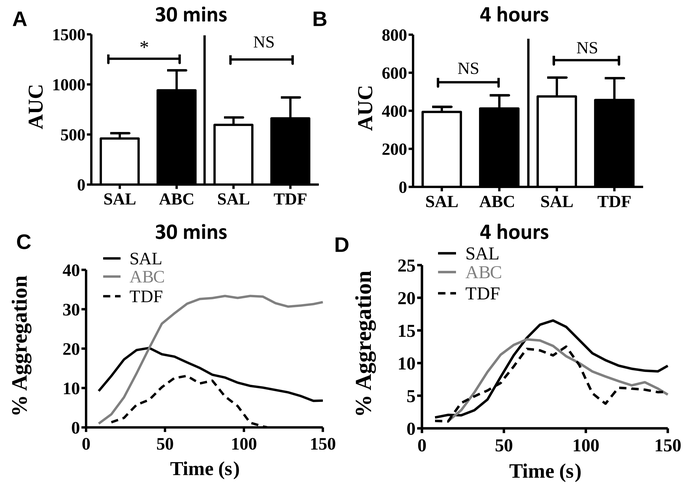


Figure 3. Effect of abacavir sulphate and tenofovir disoproxil fumarate on platelet aggregation *in vivo*.
C57bl/6 mice were intraperitoneally (*i.p.)* injected with abacavir sulphate (ABC, 30 µg/ml), tenofovir disoproxil fumarate (TDF, 30 µg/ml) or saline (SAL, 0.9 % w/v) and after 30 mins (A+C) or 4 hours (B+D) were anaesthetised (urethane 25 % w/v) and intravenously (*i.v.*) injected with collagen (50 µg/kg). Changes in the area under the curve (AUC) were measured. Data is presented as mean±SEM (A-B) or representative real-time traces (C-D), *P<0.05, NS=P>0.05 compared to SAL using a one-way test ANOVA with bonferroni post hoc analysis. n=6.

Conclusions

- **The increased cardiovascular risk associated with ABC in some studies in PLWH may be mediated by reversible pharmacological modulation of platelet and endothelial function by ABC rather than by HIV infection *per se* or differences in confounding factors between patient groups.**
- **In contrast, TDF exerts effects on platelets that would not be expected to increase the risk of platelet-driven events such as MI.**

References

[1] D:A:D study group. The Lancet. 2008; 371(9622): 1417-1426.

[2] Satchell CS, *et al*. Journal of infectious diseases. 2011; 204(8): 1202-10.