Comparative Impact of Antiretrovirals on Human Platelet Activation

Background

- Platelets are key drivers of acute cardiovascular (CV) disease
- D:A:D study\(^1\) reported a reversible increase of CV risk for abacavir sulphate- (ABC) based antiretroviral therapies (ARTs)
- Confounding issues and conflicting outcomes in clinical studies mean that increased risk cannot be directly associated with ART
- Mechanism for suggested risk is unclear but hypothesised to be driven by an effect of ART on platelet activation\(^2\)
- We employed a basic science approach to compare the pharmacological impact of ARTs, including tenofovir alafenamide (TAF), on platelet activation
- Blood from HIV-negative subjects and in-bred mice enable the impact of ART on platelets to be evaluated directly
- cGMP/NO (nitric oxide) are potent platelet inhibitors
- We explore the hypothesis that ABC (a guanosine analogue) blocks NO-mediated platelet inhibition

Aim

To investigate the comparative pharmacology of ARTs in established platelet assays to better understand ART-associated CV risk

Methods

- Blood was obtained from consenting HIV-negative volunteers
- Platelet aggregation was assessed in a 96 well plate assay allowing for comparative assessments of ARTs in blood from a single donor
- Real-time platelet granule release was assessed by flow cytometry using CD62P and CD63 antibodies
- In vivo platelet aggregation was measured in real-time in terminally anaesthetised mice

ARTs do not alter platelet aggregation in vitro

Platelets were treated in vitro with \(C_{\text{max}}\) concentrations of ART, or their active anabolites, for 30 mins. Aggregation responses to rising concentrations of ADP (left), collagen (centre) or TRAP6 (right) are shown. Max aggregation was not different from control for each ART (n=6)

ABC anabolite reverses inhibitory effect of NO

ADP-evoked aggregation was assessed in the presence of the NO donor, SNAP or vehicle. Aggregation responses were rescued by the soluble guanylate cyclase inhibitor (ODQ). Cbth-TP (A) partly reversed NO-mediated inhibition, whilst TFV (B) had no effect (n=6)

Abacavir increases platelet granule release

Collagen-evoked (10µg/mL) platelet alpha (A) and dense (B) granule release were monitored in real-time by flow cytometry. Treatment with \(C_{\text{max}}\) concentrations of ABC, but not TAF or TDF, enhanced platelet granule release. Granule release is a critical step in platelet activation (n=7)

Abacavir enhances platelet aggregation in vivo

Representative collagen-induced in vivo aggregation responses in the presence of ABC (A; n=7), TAF (B; n=3) or TDF (C; n=7). ABC treatment enhanced aggregation, whilst TAF and TDF drugs gave responses similar to vehicle control. (TAF is susceptible to plasma esterases, therefore experiments were conducted in esterase-deficient (Ces1\(^{-}\)) mice)

Conclusions

- Increased CV risk reportedly associated with ABC-based therapies may be driven, mechanistically, by enhanced platelet granule secretion and interrupted inhibition of aggregation by NO
- These effects occur independently of HIV infection and other confounding issues reported in earlier clinical studies

References:
2. Islam et al. (2012), HIV Med., 8, 453-68

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