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Changes in platelet function following abacavir administration: a pilot study

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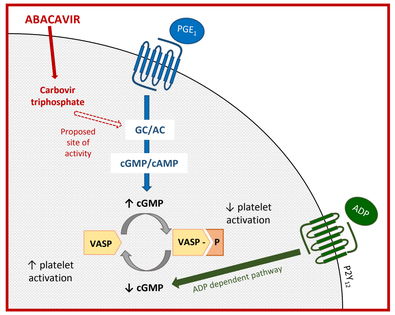
Background

- Abacavir has been associated with increased rates of cardiovascular disease (CVD) but the mechanism by which this occurs remains unknown[1]
- The association appears strongest with current exposure and the elevated risk returns to baseline within 6 months of stopping therapy
- It is physiologically plausible that alterations in platelet function may be playing a role.
- Carbovir triphosphate (the active anabolite of abacavir) may affect intra-platelet guanylyl cyclase activity leading to increased platelet activation[2]
- Switch studies have shown lower soluble glycoprotein VI (sGPVI) – a platelet specific collagen receptor - in patients who remain on abacavir compared with those who switched to tenofovir based regimens[3]

AIMS:

1. To determine if abacavir administration is associated with changes in platelet function
2. To identify if any abacavir induced changes are reversible on cessation of the drug

Figure 1: Proposed site of action of abacavir in platelets



Platelet activity is tightly regulated by complex and inter-related stimulatory and inhibitory pathways. One of these pathways operates through the phosphorylation of vasodilator-stimulated phosphoprotein (**VASP**). In the presence of cyclic guanosine monophosphate (**cGMP**) VASP is preferentially driven towards the phosphorylated state (**VASP-P**) which inhibits platelet activation. With falling levels of cGMP (such as is promoted by adenosine diphosphate (**ADP**) activity through the $P2Y_{12}$ receptor) unphosphorylated VASP predominates which promotes increased platelet activation.

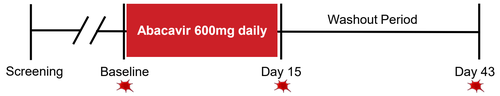
PGE_1 – Prostaglandin E_1, **GC** - guanylyl cyclase, **AC** - adenylate cyclase, **cAMP** - cyclic adenosine monophosphate

References

1. Sabin CA et al. Lancet **2008**; 371(9622): 1417-26. **2.**Baum PD et al. Aids **2011**; 25(18): 2243-8. **3.**O'Halloran JA Abstract CROI. Boston, MA, **2014**.

Methods

- An open label, single centre interventional study was performed in twenty (20) adult males, all on a stable non-abacavir containing antiretroviral (ARV) regimen with an undetectable HIV viral load
- Patients with known CVD (or whom were at high baseline risk for CVD) were excluded; as were those with a contraindication to abacavir administration or a pre-existing platelet disorder



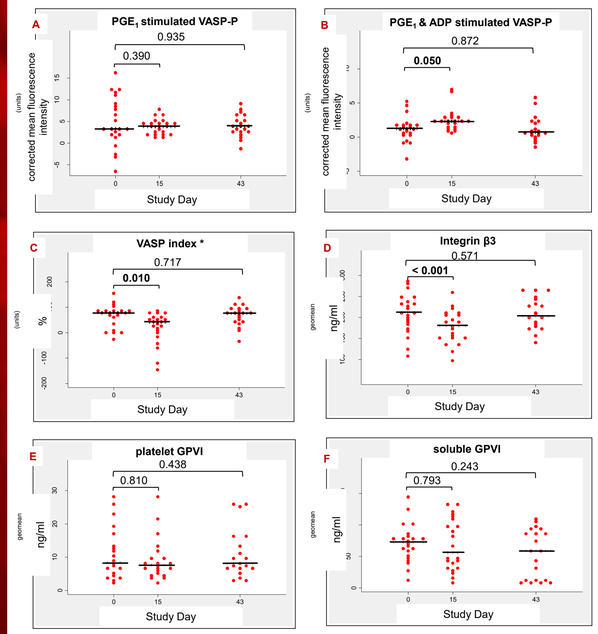
- Participants received abacavir 600mg daily for 15 days in addition to their usual ARV regimen. Plasma, serum and whole blood was collected prior to commencing abacavir, on day 15 and then again following a 28 day washout
- Flow cytometric approaches were used to measure levels of VASP-P following incubation with PGE_1 alone (resting platelets) or PGE_1 and ADP (activated platelets).
- Platelet receptors were also isolated using flow cytometric methods; GPIbα, GPVI, integrin αIIb and β3 and tetraspanin CD9; while sGPVI levels were determined by ELISA.

Table 1: Baseline Characteristics

n = 20 male participants		Median (IQR) or n (%)
Age, years		40 (30.5 – 50.5)
Ethnicity	- Caucasian - Asian	18 (90%) 2 (10%)
Duration of know HIV infection, years		6 (2 – 11)
CD4+ T cell nadir, cells/μL		270 (210 – 371)
Current CD4 + T cell count, cells/μL		660 (576 – 863)
ARV regimen	- Tenofovir/Emtricitabine - NNRTI - PI - Integrase	19 (95) 9 (45) 6 (30) 9 (45)
FRS, 10 year % risk		5 (3 – 7)
Creatinine Clearance*, ml/min		111.5 (81.2 – 127.7)
Platelet count, cells x 10^9/L		210 (177 – 233)

Results

Figure 2: Changes in VASP phosphorylation & soluble and intact platelet receptors



* VASP Index = proportional indifference in VASP-P induced by PGE_1 alone compared with PGE_1 and ADP

- Integrin β3 levels (a surrogate marker for platelet size) decreased significantly with abacavir therapy and returned to baseline following withdrawal of the drug. There was a non-significant trend towards decreased sGPVI levels while pGPVI levels were unchanged
- Integrin αIIb, GPIbα, and tetraspanin CD9 receptor levels did not change with abacavir therapy (data not shown)

Discussion

- VASP phosphorylation in the presence of PGE_1 was stable across all time points
- The de-phosphorylation of VASP promoted by ADP was decreased following 15 days of abacavir therapy; as demonstrated by a higher VASP-P concentration and lower VASP index
- The mechanism by which abacavir may be decreasing ADP responsiveness (and the clinical implication of that change) in platelets is unknown
- The observed results may be the consequence of a direct effect on the $P2Y_{12}$ receptor or represent a negative feedback mechanism responding to pro-thrombotic alterations in other pathways within the platelet or systems external to the platelet (such as alterations in endothelial function)
- Lower integrin β3 and platelet receptor levels may suggest the presence of immature platelets; and hence a megakaryocyte effect

Limitations:

- The small sample size and homogeneous study population means that the results of this pilot study need to be interpreted with caution until they have been replicated in a larger more heterogeneous group
- However they do raise doubt in the current hypothesis that abacavirs association with CVD is mediated through the guanylyl cyclase- cGMP pathway of platelet activation

Conclusions

- Short course abacavir therapy (in addition to suppressive ART) is associated with significant and reversible changes in platelet profile
- The clinical impact of these changes remains unclear
- They may represent a direct platelet effect or a negative feedback response to an unmeasured intra- or extra-platelet phenomenon

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