The role of platelet function and viability testing in addressing observations of suspected drug-induced thrombocytopenia

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Introduction

Thrombocytopenia (TP) is a side effect that can be observed in pre-clinical or clinical assessment of potential new medicines. New agents or their metabolites could induce TP by enhancing platelet consumption or the rate of platelet destruction/removal. The characteristics of TP e.g. extent, frequency of occurrence, rate of recovery of the platelet count, in combination with the results of *in vitro* assessment of the agents can be helpful in elucidating the mechanism of action and assessing the risk of their direct effects on platelets.

Conclusion

Findings using high throughput platelet assays for aggregation are consistent with the traditional assays. The plate-based formats allow for a more comprehensive assessment of the effect of agents on platelets and can be formatted to enable compound screening. Several pro-apoptotic agents in development for oncology indications have been shown to induce thrombocytopenia. We have confirmed the effects on platelet viability in line with the selectivity of their mechanism of action. The effects were also dependent on the test conditions.

Platelet function testing using higher throughput assays

Possible effects of agents on platelet function can be assessed *in vitro* using several assays to measure platelet activation or aggregation in whole blood, platelet rich plasma or isolated platelets. These assays can be performed in a 96-well plate format offering a higher throughput mode compared to traditional platelet function assays.

Platelet aggregation – isolated platelets

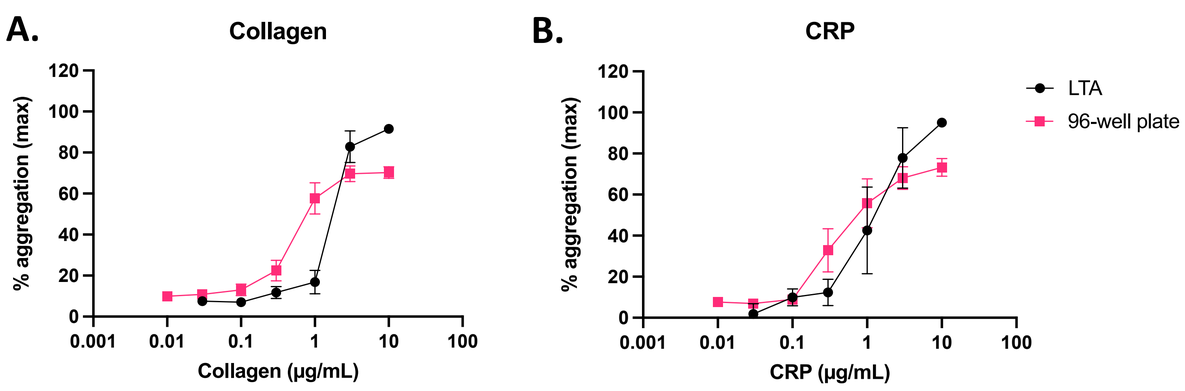


Figure 1. Platelet aggregation in isolated platelets induced by collagen (A) and collagen related peptide (CRP) (B) assessed by traditional low throughput light transmission aggregometry (LTA) (black, mean ± SEM, n=2-4) and a high throughput layout by agitation on a 96-well plate measured by light absorbance (pink, mean ± SEM, n=4).

Platelet aggregation – whole blood

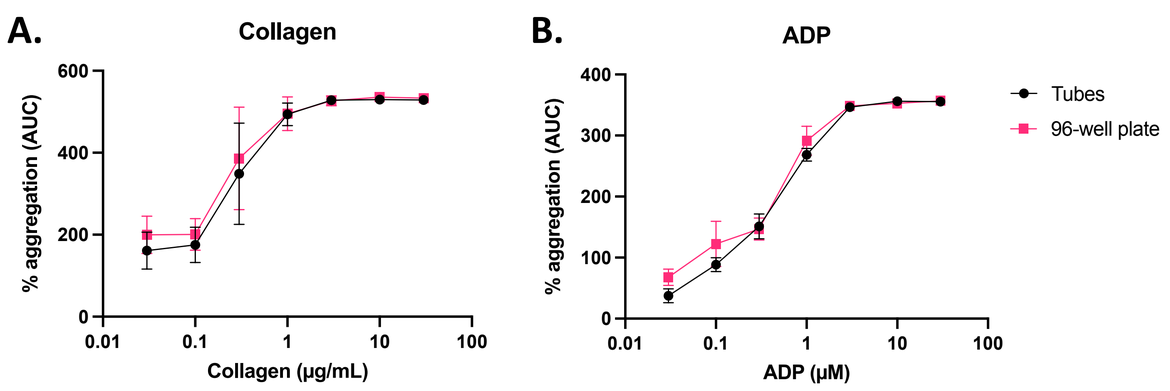


Figure 2. Platelet aggregation in whole blood induced by collagen (A) and adenosine diphosphate (ADP) (B) assessed by single platelet counting in fixed samples using a low throughput assay by stirring in tubes (black, mean ± SEM, n=4) and a high throughout layout by agitation on a 96-well plate measured by single platelet counting by flow cytometry (pink, mean ± SEM, n=3).

Platelet viability testing

The possible effects of agents on platelet viability can, in part, be addressed *in vitro* by several assays: measurement of phosphatidylserine (PS) exposure by the binding of Annexin V, metabolic activity (MTS assay) or by flow cytometry using viability dyes.

Platelet viability – assay comparison

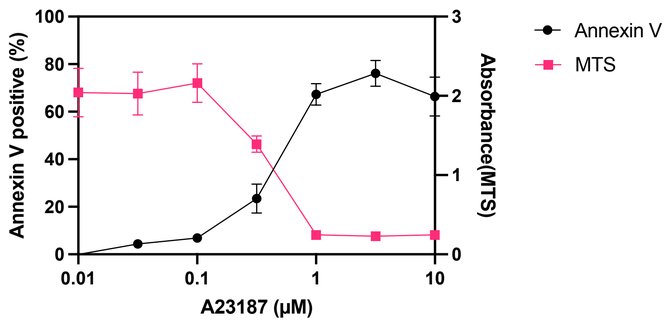
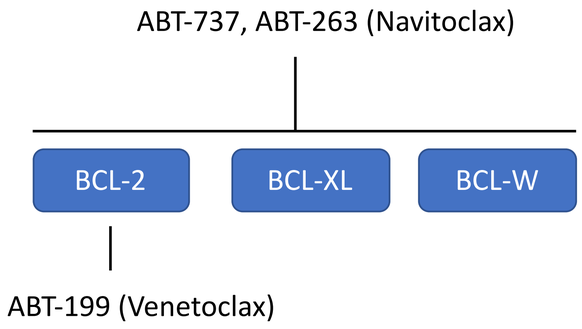


Figure 3. Viability of cells treated with calcium ionophore A23187 measured by an increase in Annexin V staining (black, mean ± SEM) and a decrease in MTS (pink, mean ± SEM), n=2.

Mode of action of some pro-apoptotic agents in development:



Effects of ABT-199 and ABT-737 on platelet viability

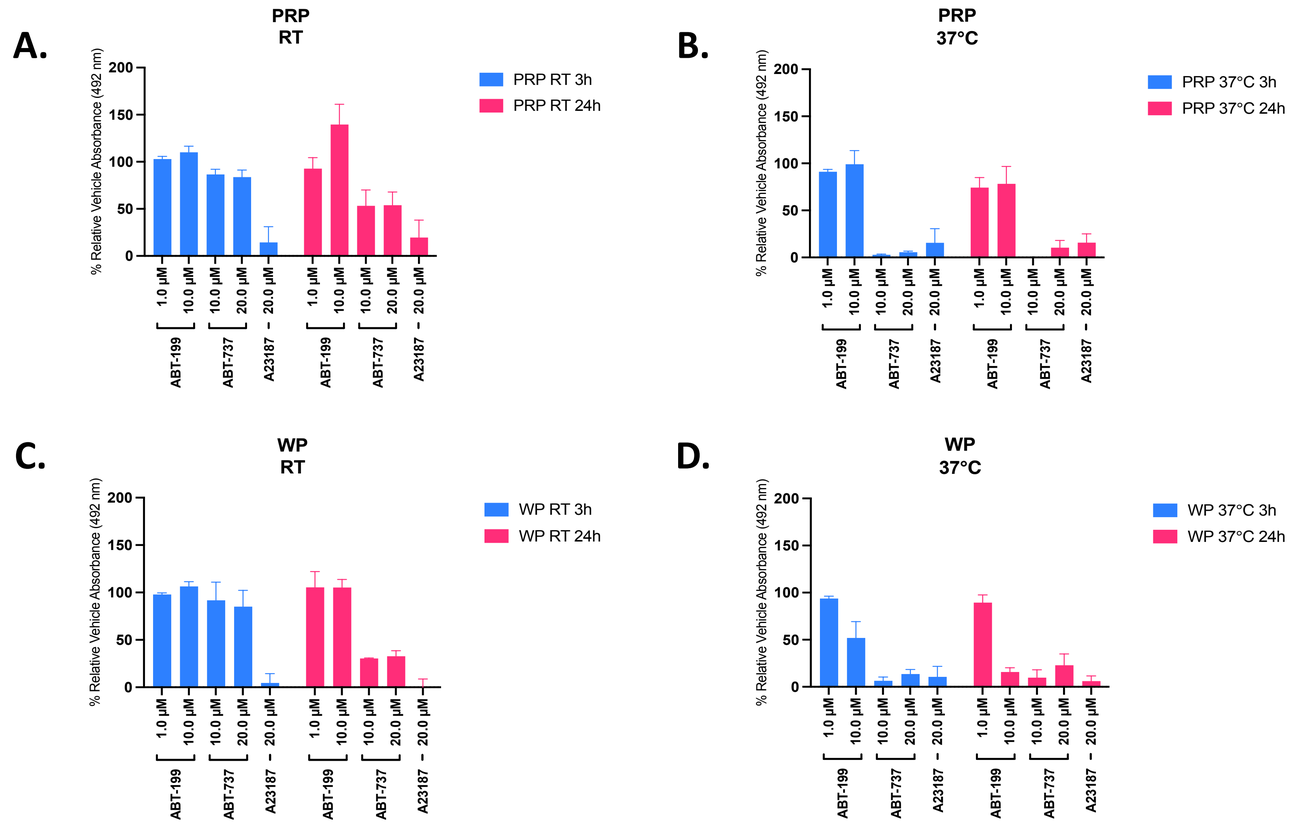


Figure 4. Viability, in platelet rich plasma (PRP) (A&B) and isolated washed platelets (WP) (C&D) treated with A23187, ABT-199 and ABT-737 measured by MTS after 3 and 24 hrs at 37˚C (B&D) or room temperature (RT) (A&C) (mean ± SEM, n=4).

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