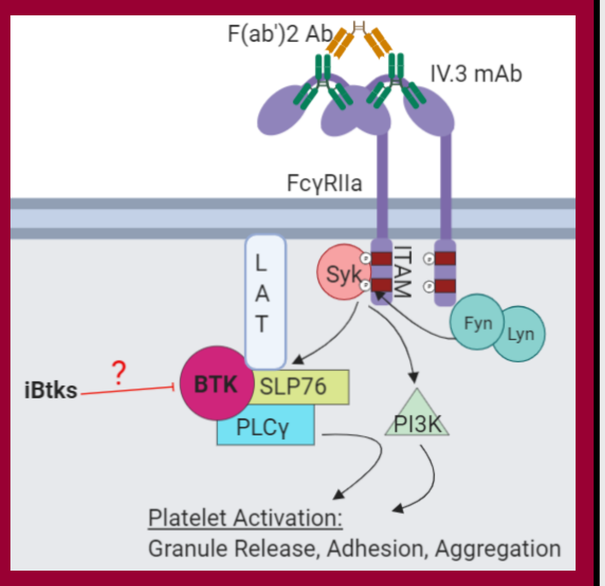
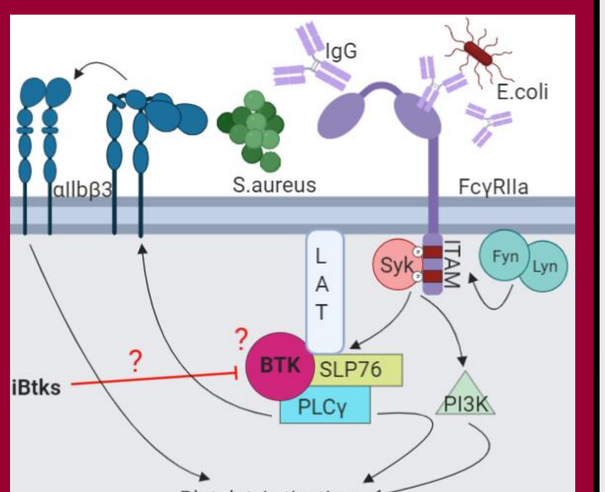
Btk Inhibitor Ibrutinib Impairs FcγRIIa-Mediated Responses of Platelets from Healthy Donors and Chronic Lymphocytic Leukaemia Patients to Bacteria

KEY POINTS

- The Bruton's tyrosine kinase (Btk) inhibitor ibrutinib is an effective treatment for chronic lymphocytic leukaemia (CLL).
- Ibrutinib is associated with increased infection risk.
- Ibrutinib inhibits aggregation to both bacteria and IV.3 mAb-mediated FcγRIIa crosslinking in healthy donor platelets and in ibrutinib-treated CLL patient platelets.
- Ibrutinib inhibits dense granule release to both IV.3 XL and bacteria in healthy donor platelets.
- Ibrutinib inhibits α-granule release to both IV.3 XL and bacteria in healthy donor and CLL ibrutinib-treated patient platelets





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INTRODUCTION

Ibrutinib is a highly effective treatment for chronic lymphocytic leukaemia (CLL), disrupting B cell receptor signalling by inhibiting Bruton's tyrosine kinase (Btk), preventing proliferation and survival signals.

Ibrutinib is associated with haemorrhagic side effects, due to the role of Btk in supporting platelet aggregation. Ibrutinib is also associated with infections in CLL patients, but the potential effects of Btk inhibitors on platelet immune function are unknown.

Platelets can be activated in response to IgG-opsonised bacteria via the platelet low affinity IgG immune receptor, FcγRIIa, of which Btk is downstream. Upon platelet activation, changes in the platelet surface and released immune mediators, including antimicrobial cytokine Platelet Factor 4 (PF4), have an impact on platelet interaction with other immune cells and pathogens.

Hypothesis: We hypothesised that Btk plays an important role in platelet FcγRIIa signalling in response to bacterial agonists, and that ibrutinib inhibits such responses, contributing towards the increased risk of infection seen in CLL.

METHODS

Isolated platelets were freshly prepared from healthy donors, ibrutinib-treated and ibrutinib-naïve and CLL patients in the presence of plasma (PRP). Healthy and ibrutinib-naïve CLL platelets were *in vitro* incubated with varying doses of ibrutinib.

Platelet activation was measured as platelet aggregation via light transmission aggregometry (LTA), α-granule secretion via a PF4 ELISA, and dense granule release via a luciferin-luciferase assay.

Crosslinked IV.3 mAb (anti-FcγRIIa), was used to directly activate the FcγRIIa receptor. Gram-positive *Staphylococcus aureus* Newman and Gram-negative *Echerichia coli* RS218 were also used, as both bacterial strains are known to cause FcγRIIa-mediated platelet activation.

RESULTS

Healthy donor PRP was incubated for 5 minutes *in vitro* with various concentrations of ibrutinib before addition of either (A) crosslinked-IV.3 mAb, (B) *S.aureus* Newman or (C) *E.coli* RS218. Platelet aggregation was measured via LTA, and shows a clear inhibition of aggregation after incubation with ibrutinib. Inhibition was not seen in response to Btk-independent thrombin receptor activator peptide 6 (TRAP6) (data not shown).

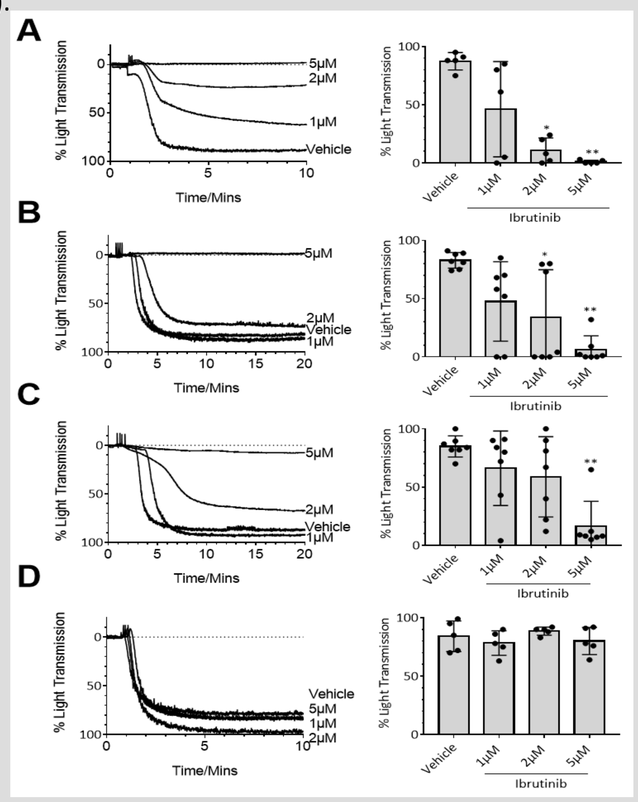

*Figure 1 - Ibrutinib inhibits platelet aggregation in response to FcγRIIa agonists in PRP. PRP was incubated for 5 minutes with ibrutinib or vehicle before the addition of (A) 4μg/ml mAb IV.3 for 2 minutes, before the addition of 30μg/ml F(ab')2 rabbit anti-mouse IgG, (B) S.aureus Newman, (C) E.coli RS218, or (D) 3 μM Trap6. Platelet aggregation was measured by light transmission aggregometry. Mean data shows ±SD. *P=0.05, **P=0.002, ***P<0.001. n=7*

Healthy PRP (A) dense and (B) α-granule release was measured via luciferin-luciferase assay and PF4 ELISA respectively after stimulation with either either (i) crosslinked-IV.3 mAb, (ii) *S.aureus* Newman or (iii) *E.coli* RS218. Use of ibrutinib is seen to inhibit granule release in response to bacteria and IV.3 crosslinking, but not TRAP6 (data not shown).

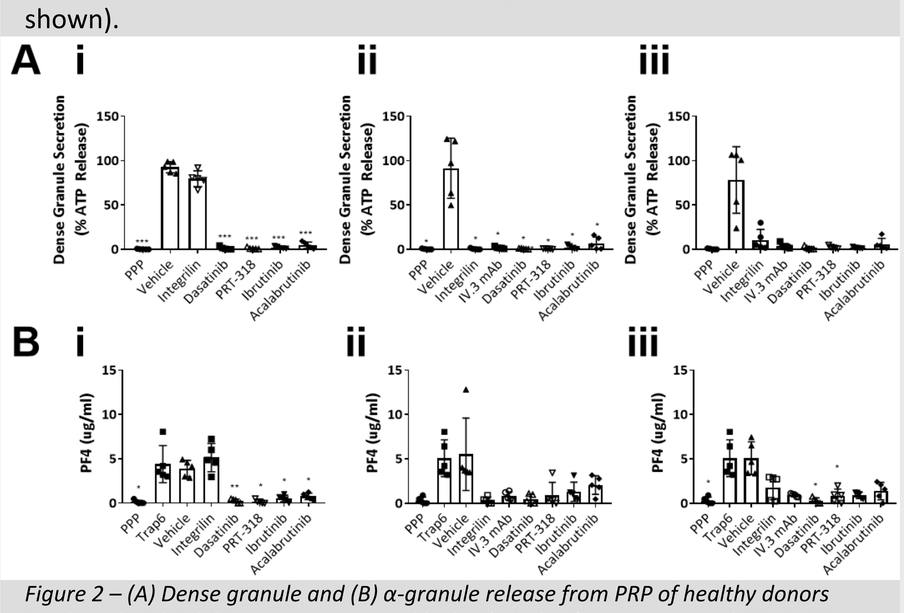

*Figure 2 – (A) Dense granule and (B) α-granule release from PRP of healthy donors incubated with inhibitors was measured by luciferin-luciferase assay and PF4 ELISA respectively, after stimulation with either (i) IV.3 XL, (ii) S.aureus Newman and (iii) E.coli RS218. Mean data shows ±SD. *P=0.05, **P=0.002, ***P<0.001. n=5*

Aggregation for ibrutinib-naïve and ibrutinib-treated CLL patients and healthy donors in PRP was measured via LTA, before a PF4 ELISA was performed for ibrutinib-treated and ibrutinib-naïve CLL PRP. Reactions were stimulated with either FcγRIIa-dependent agonists IV.3 crosslinking, *S.aureus*, *E.coli*, or FcγRIIa-independent agonists ADP, collagen related peptide (CRP) and TRAP6.

FcγRIIa-independent agonists ADP and TRAP6 gave similar responses in healthy donors, ibrutinib-treated and ibrutinib-naïve CLL platelets. CRP stimulation of ibrutinib-treated CLL PRP showed inhibition as previously seen.

Platelet aggregation and α-granule release in ibrutinib-treated CLL PRP was inhibited in response to FcγRIIa-dependent agonists IV.3 crosslinking, *S.aureus*, and *E.coli* in comparison to ibrutinib-naïve CLL PRP and healthy donor PRP.

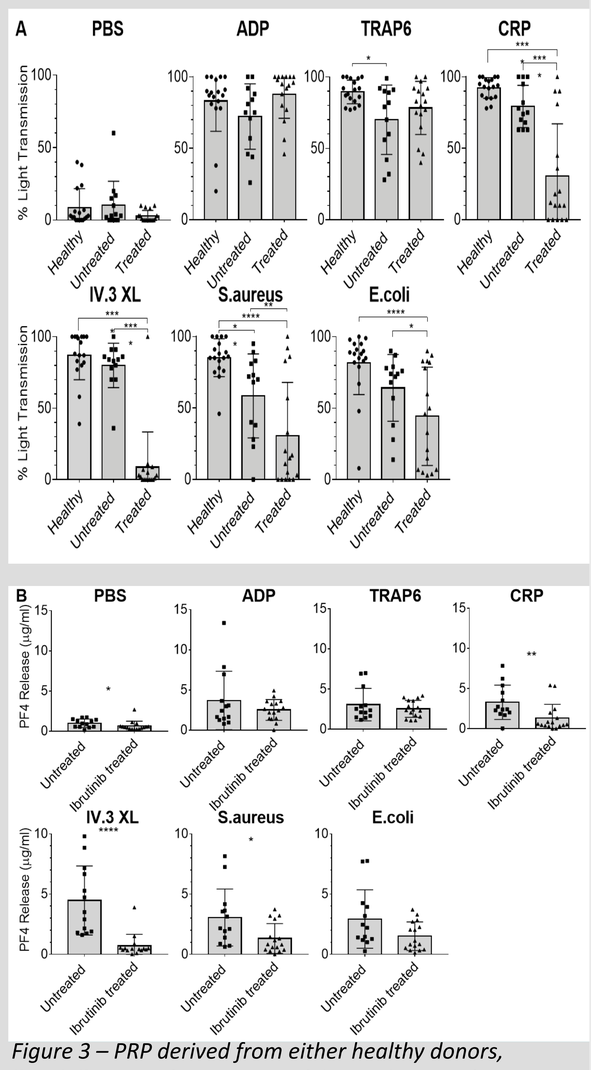

*Figure 3 – PRP derived from either healthy donors, ibrutinib-treated, or untreated CLL patients underwent either (A) LTA and (B) PF4 ELISA stimulated via IV.3 XL, S.aureus Newman or E.coli RS218, CRP, TRAP6 ADP or PBS. Mean data shows ±SD. *P=0.05, **P=0.002, ***P<0.001.*

CONCLUSIONS

These studies have demonstrated that:

- Ibrutinib-treated and ibrutinib-naïve CLL platelets are able to respond to non-FcγRIIa agonists comparable to healthy controls.
- Ibrutinib-naïve CLL PRP is able to activate in response to bacteria. This was previously unknown as CLL patients can have alterations in IgG levels and repertoires that might affect platelet FcγRIIa activation.
- Ibrutinib-treated CLL PRP has diminished aggregation and α-granule release in response to FcγRIIa agonists in comparison to ibrutinib-naïve CLL and untreated healthy donor PRP.
- Ibrutinib inhibits aggregation in healthy donor PRP to FcγRIIa agonists as well as dense and α-granule release in healthy donor PRP.

We propose that Btk inhibitor, ibrutinib, impairs the FcγRIIa pathway in platelets, leading to reduced platelet responses to bacteria, possibly contributing to the increased risk of infections seen in CLL.

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