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Low dose Btk inhibitors: an 'aspirin' of tomorrow?

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(1522 words)

The Bruton's tyrosine kinase (Btk) is one of the five members of the Tec family of cytoplasmic non-receptor protein tyrosine kinases. Expressed in hematopoietic cells, with the exception of T cells, it is essential for B cell maturation and the signal transduction of the B cell antigen receptor (BCR). The loss-of-function mutations in its gene lead to the immunodeficiency disease called X-linked agammaglobulinemia (XLA). Since the 2000s, Btk emerged as a major therapeutic target in the treatment of B-cell malignancies including chronic lymphocytic leukemia (CLL), mantle-cell lymphoma (MCL) and Waldenström macroglobulinemia.[1] Ibrutinib is the first-in-class irreversible inhibitor of Btk acting by covalent modification of the cysteine residue C418 in the kinase ATP binding domain. In the long term, its daily intake shows a remarkable activity on these malignant haemopathies.[1] Some side effects, however, include skin rashes, atrial fibrillation, hypertension, and increased risk of bleeding in about 40% of treated patients.[2] While most bleeding events are of low grade, some grade 3 or higher events are reported in 5% of patients.[2] Interestingly, in

this issue of Haematologica, Nicolson and colleagues propose a therapeutic application for Btk inhibitors at low doses in thrombosis.[3]

Two members of the Tec family expressed in platelets, Btk and Tec, are involved in signal transduction downstream of platelet receptors containing an immunoreceptor tyrosine-based activation motif (ITAM) or a closely related hemITAM.[4] Glycoprotein VI (GPVI), a platelet specific collagen receptor constitutively associated with the ITAM-containing FcRγ-chain, uses Btk to phosphorylate phospholipase Cγ2 (PLCγ2).[5] PLCγ2-mediated calcium mobilization and protein kinase C activation are critical for collagen-evoked platelet activation and aggregation.[4] Tec can partially compensate for the absence of Btk downstream of GPVI.[6] The hemITAM-containing C-type lectin-like receptor 2 (CLEC-2), a podoplanin receptor highly expressed in platelets, also uses Btk and Tec.[4,7] Finally, the platelet-specific receptor for von Willebrand factor, GPIb, which contains neither ITAM nor hemITAM, activates Btk.[8]

Following the observation of an increased bleeding risk in patients treated with ibrutinib, several studies have demonstrated that the drug affects platelet functions *in vitro* and *ex vivo* through both on- and off-target effects.[2,9-12] Consistent with the fact that XLA patients do not experience increased bleeding, ibrutinib-related haemorrhagic syndrome in patient with B-cell malignancies would be mainly due to off-target effects of the drug. [12-14] Ibrutinib has for instance been shown to affect Src-family kinases (SFK) in platelets. [12-14] The relatively high dosing of ibrutinib in CLL (420 mg/day) and MCL (560 mg/day) treatment likely contributes to the platelet effect via inhibition of Btk, Tec and also off-targets such as SFK. Second-generation Btk inhibitors with greater specificity for Btk, including acalabrutinib and tirabrutinib,[1] have a lesser impact on platelets and, compared to ibrutinib, reduce the risk of severe bleeding in treated patients. [2,12-14]

They do not abolish the mild bleeding diathesis that may be influenced by intrinsic features of B-cell malignancies in addition to the anti-platelet effect of the drugs.[2,12-14]

Nicolson and colleagues report that low doses of Btk inhibitors selectively block platelet activation dependent on CLEC-2. [3] Such low doses only delay platelet aggregation induced by GPVI triggering. The authors propose that the differential effect of Btk inhibition in CLEC-2, relative to GPVI signalling, is due to a positive feedback loop involving thromboxane A2 (TXA2) and adenosine diphosphate (ADP). This important Btk-dependent positive feedback control from TXA2 and ADP receptors observed in human platelets stimulated through CLEC-2 was not found in mouse platelets. Accordingly, mouse platelets require higher concentrations of ibrutinib to block CLEC-2-mediated activation. Furthermore, contrasting with the conclusion of a previous study,[7] the authors convincingly demonstrated that the Syk tyrosine kinase lies upstream of Btk in the CLEC-2 signalling cascade. The mandatory role of Btk downstream of CLEC-2 was confirmed by using platelets from XLA patients, which were unable to aggregate and phosphorylate PLCγ2 on the Btk phosphorylation site in response to CLEC-2 activation. Interestingly, Tec was not able to compensate for the lack of Btk in the CLEC-2 signalling. Nicolson et al.[3] also show that while Btk is important through both its kinase activity and its docking protein properties downstream of GPVI, only its kinase activity is critical for platelet activation by CLEC-2.

As an application of their observation, Nicolson and colleagues [3] proposed that low dose of ibrutinib could prevent deep vein thrombosis (DVT) as platelet CLEC-2 was previously shown to play a key role in this pathology.[15] This hypothesis was tested in a mouse experimental model of DVT, known to be dependent on platelet CLEC-2. In the group of 13 mice treated with ibrutinib (35-70 mg/kg) a trend towards reduction of inferior vena cava thrombus prevalence was observed compared to the control group. The authors suggest that the lack of statistical significance may result from an incomplete inhibition of CLEC-2

signalling through Btk pharmacological blockade within the 48 hours required for venous thrombosis in this experimental model, and from the fact that mouse platelet CLEC-2 is less critically dependent on Btk than human platelet CLEC-2. Among the small number of mice that formed a thrombus despite ibrutinib treatment, the thrombus size was comparable to the control group, suggesting that CLEC-2 could primarily act in the thrombus initiation phase.

Considering the critical role of CLEC-2 in DVT [15] as well as in bacterial-infection-induced thrombo-inflammation, [16] and its weak contribution to normal haemostasis,[4] these results suggest that a low concentration Btk inhibitors could be an option to prevent thrombotic complications in these situations.

The study of Nicolson and colleagues [3] is the second example showing that low-dose Btk inhibitors could be considered as potential antithrombotic drugs. Recently, it was reported that low doses of Btk inhibitors, including ibrutinib and the novel irreversible inhibitors, prevent platelet thrombus formation on human atherosclerotic plaques obtained from carotid endarterectomy, at arterial blood flow rates.[17] The authors suggest that this atherosclerotic plaque-selective platelet activation was blocked due to the targeting of signal transduction of GPVI (strongly activated by plaque collagen), and to a lesser extent GPIb, by Btk inhibitors. Optimization of GPVI signalling inhibition by Btk inhibitors led to the demonstration that low blood concentrations of inhibitors are sufficient for GPVI-selective platelet inhibition relevant for atherothrombosis, while sparing primary haemostasis.[18] This is consistent with the fact that, like CLEC-2, GPVI has a minimal role in normal haemostasis. [4] Through a small pilot study, the authors could show that ibrutinib at low dose (140 mg/day or each two days for a week) was sufficient to prevent atherosclerotic plaque-induced platelet thrombus formation under flow *ex vivo* and was more effective than aspirin (100 mg/day).[17-19]

Whether CLEC-2-mediated Btk activation is also targeted in the prevention of atherothrombosis by Btk inhibitors was not investigated in these studies. However, since

podoplanin (which is not expressed in the normal vascular wall) has been found in atheromatous plaque, it is tempting to speculate that blockade of CLEC-2/Btk axis also contributed to this effect. [20] This could explain the high efficacy of Btk inhibitors at relatively low dosing in this condition. Finally, a recent study has reported that ibrutinib treatment could reduce platelet-mediated inflammation contributing to atherogenesis in an obese rhesus macaque model of early atherosclerosis (Kohs T. et al. ISTH 2020 Congress abstract, Res Pract Thromb Haemost 2020;4 (Suppl 1)).

In conclusion, Nicolson and colleagues [3] bring important new information on the Btk contribution in platelet CLEC-2 signalling cascade and propose that targeting Btk downstream of CLEC-2 could protect against DVT and thrombo-inflammation. This study alongside those from Busygina and colleagues [17-19] and Kohs and colleagues shed light on the potential application of Btk inhibitors in vascular and cardiovascular diseases. Considering the very active development of Btk inhibitors in cancer treatment, such tyrosine kinase inhibition-based antiplatelet therapy, rather than more conventional approaches targeting platelet specific surface receptors, is attractive. One can however question whether the exciting results summarized above are a sufficient proof of concept to open Btk inhibitors a new therapeutic orientation. The fact that GPVI/Btk and CLEC-2/Btk axis are weakly involved in normal haemostasis and more strongly implicated in thrombotic complications such as DVT, thrombo-inflammation and atherothrombosis is a strong argument. However, several issues remain to be solved before undergoing clinical trials to evaluate the efficacy of low doses Btk inhibitors compared to current antithrombotic treatments in these pathologies. For example, do we need to inhibit both Btk and Tec to obtain an antithrombotic effect in the different situations? Do low doses Btk inhibitors: *i)* inhibit preferentially Btk in platelets and spare B cell functions, *ii)* prevent thrombus formation *in vivo* in other relevant animal models

of DVT, thrombo-inflammation and atherothrombosis such as rabbit and *iii)* spare homeostasis of the lymphatic system?

More clinical questions would also be useful to tackle such as *i)* the potential relative protection of XLA patients and oncology patients under Btk inhibitors against DVT and atherothrombotic complications, *ii)* the impact of low doses Btk inhibitors on the side effects observed with long-term treatment at high doses ibrutinib such as atrial fibrillation, or *iii)* the potential efficacy of reversible Btk inhibitors to prevent thrombotic events in short-term application. Let us bet that future studies will resolve these issues as such a new treatment is of obvious appeal in the prevention of thrombosis in different pathological situations.

Conflict of interest

The authors declare no conflict of interest.

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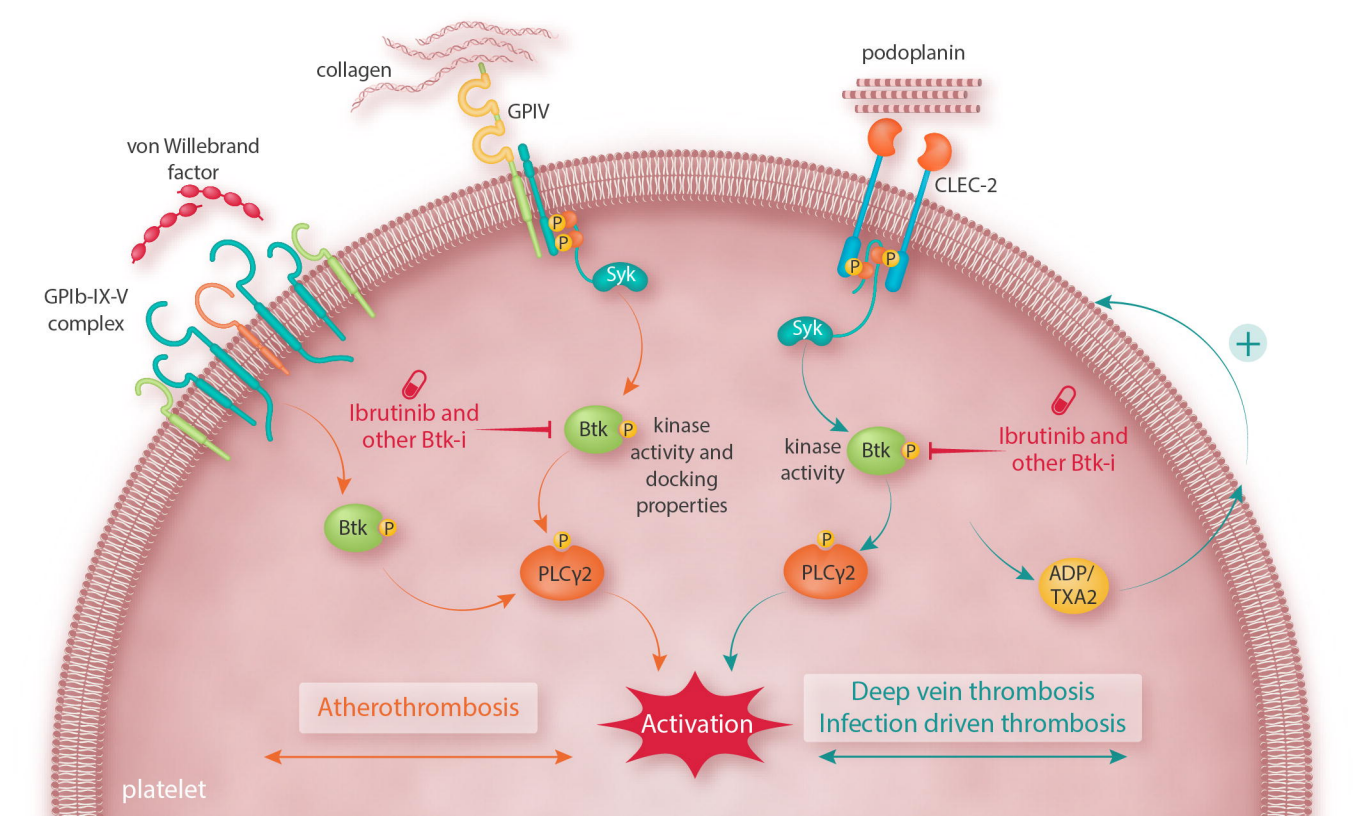
Figure legend

Figure 1. Btk inhibition downstream of platelet GPVI and CLEC-2 as a new strategy to prevent DVT, thrombo-inflammatio and atherothrombosis.

This schematic and simplified representation highlights the role of Btk in the intracellular signalling pathways evoked by platelet receptors such as GPVI, GpIb-IX-V and CLEC-2. Low doses of Btk inhibitors, compared to the relatively high doses currently used for treatment of B-cell malignancies, strongly prevent CLEC-2-evoked platelet activation and may protect against DVT and infection-driven thrombosis. The Btk-dependent positive feedback loop involving ADP and TXA2 in human platelets stimulated by CLEC-2 triggering is shown. Moreover, inhibiting Btk downstream of GPVI (strongly triggered by plaque collagen), and to a lesser extent GpIb, may protect against atherothrombosis.

Low doses irreversible inhibitors may be more effective on platelets that lack *de novo* synthesis of Btk than on nucleated cells such as B cell. It will however be important to determine the appropriate dose of Btk inhibitors to use, as some individuals are more sensitive than others.[14]

Abbreviations: GP: glycoprotein; PLC: phospholipase C; VWF: von willebrand factor, Syk: spleen tyrosine kinase; Btk: Bruton's tyrosine kinase; CLEC-2: C-type lectin-like receptor 2.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Activation
Deep vein thrombosis
Infection driven thrombosis
platelet