Effect of ibrutinib on platelet aggregation

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SUMMARY

Objective: We aimed to evaluate the in vitro impact of ibrutinib on platelet aggregation.
Methods: Light transmission aggregometry was performed using the international protocol for the laboratory investigation of platelet function. Platelet aggregation was assessed using the PACKS-4 aggregometer. Each sample was tested with adenosine diphosphate (ADP) (2.5 μmol/L), epinephrine (EPI) (1.2 μmol/L), arachidonic acid (AA) (1.0 mmol/L), and collagen (1.25 μg/mL).
Results: We tested 7 relapsed/refractory CLL patients, median age 69 years (range 52–80 years), 5 of which were aged 65 years or older. All patients received 420 mg oral ibrutinib daily. As expected, patients on ibrutinib showed impairment of response to collagen. All patients showed reduced platelet aggregation after AA. In addition, the majority of patients also had a reduced response to EPI and ADP.
Conclusion: We suggest that ibrutinib inhibits in vitro collagen and AA-induced platelet aggregation in CLL patients without any other antiplatelet therapy. We also present certain evidence that ibrutinib affects in vitro ADP and EPI-induced aggregation in a small group of CLL patients without any other antiplatelet therapy. Our findings may have some important clinical implications given that we have shown ibrutinib to affect platelet activation and aggregation on many levels. These data are preliminary and will have to be confirmed in future studies.

KEY WORDS

aggregation – bleeding – ibrutinib – platelet

SÚHRN

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Vplyv ibrutinibu na doštičkovú agregáciu
Cieľ: Cieľom práce je zhodnotiť in vitro vplyv ibrutinibu na doštičkovú agregáciu.
Metódy: Na meranie doštičkovej agregácie sme použili optickú agregometriu, pričom sme použili medzinárodný odporúčaný protokol. Na meranie sme použili PACKS-4 agregometer. Každá vzorka bola testovaná adenozíndifosfátom (ADP) (2,5 μmol/l), adrenalínom (EPI) (1,2 μmol/l), kyselinou arachidónovou (AA) (1 mmol/l) a kolagénom (1,25 μg/ml).
Výsledky: Do štúdie bolo zaradených 7 pacientov s relabujúcou/refraktérnou chronickou lymfocytovou leukémiou (CLL) s mediánom 69 rokov (rozsah 52-80 rokov); 5 pacientov malo vek 65 a viac rokov. Všetci pacienti dostávali v tabletkovej forme 420 mg ibrutinibu denne. Ako sme predpokladali u pacientov sme pozorovali nižšiu agregáciu po kolagéne. U všetkých pacientov sme pozorovali aj nižšiu odpoveď po AA. Navyše, u väčšiny pacientov sme pozorovali aj zníženú agregáciu po EPI a ADP.
Záver: Predpokladáme, že ibrutinib inhibuje in vitro spostredkovanú agregáciu kolagénom a AA u pacientov s CLL bez inej antiagregačnej liečby. Ukázali sme tiež, že ibrutinib u časti pacientov s CLL a bez inej antiagregačnej liečby inhibuje in vitro agregáciu sprostredkovanú cez ADP a EPI. Naše výsledky môžu mať významné klinické dôsledky, pretože sme ukázali, že ibrutinib ovplyvňuje doštičkovú agregáciu na mnohých úrovniach. Tieto údaje sú predbežné a budú musieť byť potvrdené v ďalších štúdiách.

KĽÚČOVÉ SLOVÁ

agregácia – krvácanie – ibrutinib – trombocyty

INTRODUCTION

Ibrutinib is an inhibitor of Bruton's tyrosine kinase (BTK). BTK is a cytoplasmic tyrosine kinase of the TEC family that is essential for B-cell receptor signalling. It demonstrates activity in chronic lymphocytic leukaemia (CLL). Ibrutinib is generally well tolerated but is associated with an increased risk of bleeding [1, 2]. Understanding the mechanism by which ibrutinib may contribute to bleeding is important for clinical management because the majority of CLL patients are elderly and many of them have comorbidities requiring anticoagulation and/or antiplatelet therapy. It is assumed that the bleeding events are caused by platelet dysfunction [3]. However, previous studies have yielded variable results. Farooqui et al. tested samples from ibrutinib-treated patients using the PFA-100 device with epinephrine (EPI) and adenosine diphosphate (ADP); no functional abnormalities were identified [4]. Until now, the best paper on this topic was published by Kamel et al. [3]. He showed (n = 23 patients) a specific and reversible effect on collagen-induced platelet aggregation. He claimed that he found no evidence of impaired ADP, EPI or arachidonic acid (AA) response. On the other hand, he showed in his graphs that a proportion of patients who had received ibrutinib had reduced aggregation after AA and EPI. Unfortunately, these findings were not discussed in the paper itself. We therefore aimed to evaluate the *in vitro* impact of ibrutinib on platelet aggregation.

MATERIAL AND METHODS

This study was approved by the local Ethics Committee. All study participants agreed to take part in the project and signed a written informed consent in accordance with the Declaration of Helsinki. Light transmission aggregometry (LTA) was performed using the international protocol for the laboratory investigation of platelet function [5]. Antecubital venous blood was collected into tubes containing 3.2% buffered sodium citrate (anticoagulant-blood ratio 1:9) to assess platelet aggregation. Platelet aggregability was tested with platelet-rich plasma using platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories, USA). Each sample was tested with ADP (2.5 µmol/L), EPI (1.2 µmol/L), AA (1.0 mmol/L), and collagen (1.25 µg/mL). Based on our own laboratory assessment of normal subjects, we defined the upper and lower normal values of aggregometry (the reference range is 55–90%). Testing was performed on patients without any antiplatelet therapy (10–14 days before measurement) and when the platelet counts had improved sufficiently following ibrutinib therapy to permit reliable testing ($\geq 100 \times 10^9$/L). All patients had normal basic coagulation tests (prothrombin time, thrombin time, activated partial thromboplastin time). Testing was performed within one hour of taking ibrutinib.

RESULTS

We tested 7 relapsed/refractory CLL patients, median age 69 years (range 52–80 years), 5 of which were aged 65 years or older. Five patients had received ≥ 2 previous lines of treatment. All patients received 420 mg oral ibrutinib daily. Median time from ibrutinib initiation to platelet aggregometry was 171 days (range 80–272 days). Median platelet count from whole blood was 117 x 10^9/L (range 103–195 x 109/L). None of these patients received antiplatelet or anticoagulation treatment. Clinical bleeding while taking ibrutinib did not occur.

The results of LTA for subjects on stable ibrutinib therapy are shown in Figure 1 and Table 1. As expected, patients on ibrutinib showed impairment of response to collagen. The maximum aggregation value was 44% and the minimum 3.86%. However, we did not expect the results after aggregation with AA, EPI and ADP. All patients showed reduce platelet aggregation after AA (maximum 52.28% and minimum 30%). In addition, the majority of patients also had a reduced response to EPI (maximum 61% and minimum 30%) and ADP (maximum 71.67% and minimum 20%).

DISCUSSION

This study reports the use of the sensitive LTA method to assess platelet function in subjects treated with ibrutinib. We showed an effect on collagen and AA--induced platelet aggregation, and some evidence of an effect on ADP and EPI-induced aggregation. We asked ourselves, "Is there any connection between collagen,

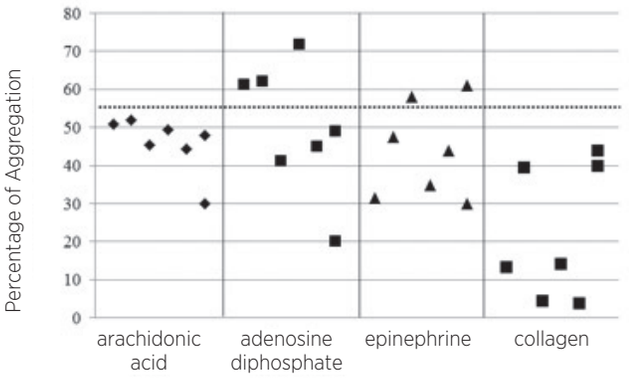

Fig. 1. LTA in samples taken from patients on stable ibrutinib therapy The percentages of peak responses for each of the different platelet agonists are shown.

Table 1. LTA results from patients on stable ibrutinib therapy

Inductor	1st patient	2nd patient	3rd patient	4th patient	5th patient	6th patient	7thpatient
Arachidonic acid (aggregation, %)	50.9	52.28	45.45	49.39	44.28	30	48
Adenosine diphosphate (aggregation, %)	61.27	61.99	41.13	71.67	44.89	20	49
Epinephrine (aggregation, %)	31.53	47.48	58	34.76	44	30	61
Collagen (aggregation, %)	13.4	39.39	4.45	14.23	3.86	40	44

ADP, EPI and thromboxane A^2 (TXA^2) receptors?" In the next section, we will try to find the answer.

Activation of glycoprotein VI (GPVI) by collagen results in a complex cascade of signalling events [6]. The initial step is phosphorylation of the FcR -chain by Fyn and Lyn. Subsequent activation of the tyrosine kinase Syk leads to phosphorylation of multiple signalling proteins, including BTK. The activated BTK then phosphorylates phospholipase C (PLC), leading to its activation. The activated PLC converts phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messenger inositol 1,4,5-trisphosphate (IP3), which in turn binds to IP3 receptors (IP^3R) located on the endoplasmic reticulum (ER). Binding of IP3 to the IP^3R is essential for triggering a calcium release from the ER [7].

Receptors for ADP, EPI and TXA2 belong to G protein-coupled receptors (GPCRs). GPCRs interact with the trimeric G-protein alpha-s/beta/gamma complex and trigger the exchange of GDP to GTP bound to G-protein alpha subunits leading to the dissociation of beta/gamma heterodimers. G-protein beta/gamma signalling also regulates phosphoinositide metabolism by increasing the kinase activity of BTK, a known activator of PLC or by direct activation of PLC [8].

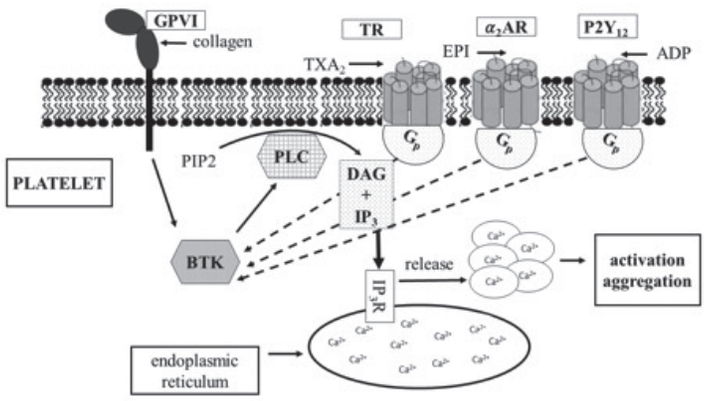

Fig. 2. PLC activation via BTK
Abbreviations: AA – arachidonic acid; α^2AR - α^2-adrenergic receptor; ADP – adenosine diphosphate; BTK – Bruton's tyrosine kinase; Ca^{2+} – calcium ions; DAG – diacylglycerol; EPI – epinephrine; Gp – G protein; GPVI – glycoprotein VI; IP^3 – inositol 1,4,5-trisphosphate; IP^3R – inositol 1,4,5-trisphosphate receptor; PIP2 – phosphatidylinositol 4,5-bisphosphate; PLC – phospholipase C; TR – thromboxane receptor; TXA^2 – thromboxane A^2.

Now, we see that the common activation site of PLC is BTK, see Figure 2. On the other hand, GPCRs can directly activate PLC. Therefore, we assume that the differences in results between studies are also affected by this fact. Our findings could have certain important clinical implications because platelet activation and aggregation is affected at many levels. Nevertheless, reduction or absence in collagen-induced platelet aggregation appears to be largely dispensable in normal life, presumably due to activation in other platelet pathways [3]. However, the situation changes dramatically during concomitant administration of antiplatelet or anticoagulant agents. Concomitant use of these drugs should be instituted with utmost care and only when really necessary.

There were several limitations in our study, including the small number of patients and platelet count during aggregometry. Currently, only 12 patients with CLL are treated with ibrutinib in Slovakia. Platelet aggregability is greatly affected by preanalytical issues and therefore interpretation of platelet aggregability may be potentially adversely affected. Therefore, we follow the international protocol [7].

CONCLUSION

We showed an effect on collagen and AA-induced platelet aggregation and some evidence of an effect on ADP and EPI-induced aggregation. Our findings may have some important clinical implications given that we have shown ibrutinib to affect platelet activation and aggregation on many levels.

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Conflict of interest
The authors declare no conflict of interest.

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Author contribution
JS, JC and IS collected and analysed data. All authors reviewed the literature and wrote the manuscript.

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