performed the analyses; S.N. and D.M. wrote the manuscript, which was critically revised by M.S., L.B., J.J. and B.R.; and data interpretation was performed by all coauthors.

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To the editor:

Bcl-xL–inhibitory BH3 mimetic ABT-737 depletes platelet calcium stores

Recently, several studies have been published in Blood that have examined the effect of Bcl-xL–inhibitory BH3 mimetics on platelet function and lifespan.1,3 These reports agree that 2 related BH3 mimetics, ABT-737 and ABT-263, induce platelet apoptosis. This is characterized by caspase-3 activation and caspase-dependent phosphatidylserine externalization, and may underlie the thrombocytopenia observed during administration of these drugs.2,4 However, in addition, the study by Vogler et al made 2 specific observations regarding the effect of BH3 mimetics on platelet calcium signaling: first, that they induce a transient calcium signal; and second, that prolonged treatment with BH3 mimetics depletes intracellular calcium stores.1 In contrast, Schoenwaelder and Jackson, in correspondence to the editor, dispute these observations.5

We have also investigated the effect of ABT-737 on platelet calcium signaling. In agreement with Vogler et al, we find that ABT-737 (10 µM) can induce a calcium signal (Figure 1A). However, the signal that we detect is small relative to that induced by thrombin. The maximum increase in cytosolic calcium concentration above basal was estimated as ~50 nM, and was variable between platelets from different donors. For clarity, we have

![Figure 1. Effect of ABT-737 on platelet calcium signaling.](image-url)

- **Figure 1. Effect of ABT-737 on platelet calcium signaling.** (A) Washed platelets loaded with Fura-2 (1 × 10^7/mL) were treated with ABT-737 (10 µM; gray trace) or DMSO (black trace) in the presence of extracellular CaCl_2 (1 mM), followed by stimulation with thrombin (0.1 U/mL). The initial section of the traces are expanded and separated below to show the small ABT-737–induced signal. Fluorescence was calibrated in terms of cytosolic calcium concentration to estimate the magnitude of the response. (B) Platelets in modified Tyrode buffer were treated with ABT-737 (10 µM) or DMSO or for the indicated time then treated with EGTA (1.2 mM) followed by ionomycin (Iono; 1 µM) to artificially deplete calcium stores. Traces are representative of at least 3 independent experiments. All experiments were performed at 37°C under stirring conditions.
expanded this section of the trace to show the ABT-737–induced calcium signal. This small signal might not be sufficient to directly activate platelets, although it could reflect a larger but more local subcellular signal, or a subpopulation of platelets that respond differently compared with most platelets.

We also found that incubation with ABT-737 induced calcium store depletion. However, this was not a rapid event, but rather required prolonged incubation with ABT-737. To assess Ca\(^{2+}\) store content, we treated platelets with 10\(\mu\)M ABT-737 for either a short time (5 minutes) or a long time (120 minutes). The extracellular Ca\(^{2+}\) was then chelated by EGTA (1.2mM) and ionomycin (1\(\mu\)M) added to artificially release the intracellular calcium stores (Figure 1B). Brief treatment with ABT-737 (5 minutes) did not substantially affect the ionomycin-induced Ca\(^{2+}\) signal, indicating that the filling state of the intracellular Ca\(^{2+}\) stores was unaffected. This is consistent with the observations reported by Schoenwaelder and Jackson. However, ionomycin-induced Ca\(^{2+}\) release was substantially reduced after longer treatment with ABT-737. This suggests that the ionomycin-sensitive intracellular Ca\(^{2+}\) stores are slowly depleted after inhibition of Bcl-X\(_L\), consistent with the data of Vogler et al.

Taken together, our data indicate that BH3 mimetics such as ABT-737 modify intracellular Ca\(^{2+}\) handling in platelets. Whether this contributes to platelet apoptosis and BH3 mimetic–induced thrombocytopenia requires further investigation.

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To the editor:

Platelet tissue factor is not expressed transiently after platelet activation

Blood coagulation is initiated by the binding of plasma factor VII/VIIa to cell surface–expressed tissue factor (TF). Normally, TF expression is confined to subendothelial tissues like fibroblasts and pericytes to limit blood coagulation to sites of vascular injury. Consistent with this notion, our laboratory demonstrated previously that platelets do not express active TF constitutively, or as a result of de novo synthesis, transfer from monocytes, or \(\alpha\)-granule release. Recently, Camera et al suggested that expression of TF by platelets occurs rapidly after activation, and is transient, as expected from detectable TF antigen and activity after a 2-hour platelet stimulation. In that study, 3 different anti-TF antibodies (2 obtained from commercial sources and another obtained from Dr Y. Nemerson, Mt Sinai School of Medicine, New York, NY [deceased]) were used and TF antigen was detected on the platelet surface after activation with a 6-amino acid protease activated receptor-1 (PAR1) agonist peptide for 15 minutes. Using a commercially available antibody (American Diagnostica; 45-07CJ), an ~50% increase in the mean fluorescence intensity (MFI) of the immunostained, activated platelets was observed when compared with unactivated platelets, or when activated platelets were stained with an isotype-matched control antibody. In contrast, no TF expression (< 10% increase in MFI) was observed after a 2-hour stimulation, consistent with our previous observation. In an attempt to duplicate these results, we performed analogous experiments using 2 different fluorophore-conjugated anti-TF antibodies including a specific, inhibitory anti-TF monoclonal antibody described previously (anti–TF-5) and the same commercially available anti-TF antibody used by Camera and colleagues (American Diagnostica, 45-07CJ). By flow cytometry, nearly identical levels of anti-TF-5 immunoreactivity with platelets, activated for 15 minutes (728 ± 118.3 MFI; dotted line) or 2 hours (625.3 ± 62.3 MFI; black line) with PAR 1 agonist peptide (SFLLRN), were obtained (Figure 1A). Interestingly, we observed similar results using the same anti-TF antibody used by Camera and colleagues. No difference in MFI between 15 minutes (5263 ± 683.5 MFI) and 2 hours (5426 ± 646.5 MFI) of platelet activation was observed (Figure 1B dotted and black lines, respectively). In both instances, the MFI were similar to those obtained when platelets were immunostained using isotype-matched control antibodies (753.8 ± 78.9 MFI and 4543 ± 568.6 MFI; respectively; shaded histograms). Control experiments performed using a lipopolysaccharide-stimulated monocyte-like cell line (THP-1) versus unstimulated cells confirmed positive immunoreactivity of the antibodies. In the absence of LPS-stimulation, few cells expressed TF (4.3 ± 0.1%) using anti–TF-5 (Figure 1C dotted line). Subsequent to LPS-stimulation, 49.3 ± 0.0% of the cells

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