required to trigger the immediate calcium response. The precise reason that Schoenwaelder and Jackson did not observe an increase in cytosolic free calcium at higher concentrations of ABT-263 is unclear but may well relate to differences such as higher platelet density (3 × 10^8 cells/mL used by them compared with the lower cell density 5 × 10^7 cells/mL used in our study). In this regard we have previously reported that the efficacy of both ABT-263 (Navitoclax) and ABT-737 in inducing apoptosis of primary chronic lymphocytic leukemia (CLL) cells is markedly diminished both by increasing cell density as well as by protein binding.

Schoenwaelder and Jackson assessed the calcium response after only 10 minutes of pretreatment with ABT-737. In our initial study, the depletion of cellular calcium stores was assessed only after exposure to ABT-263 or ABT-737 for 2 hours. This time point was selected because we did not consistently detect caspase activation earlier and wished to investigate the contribution of caspases to the depletion of intracellular calcium stores. We can only hypothesize that 10 minutes of treatment with ABT-737 is not sufficient to deplete the intracellular calcium stores, thus explaining the obvious difference in our results.

Taken together, our data consistently show that the BH3 mimetics, ABT-263 and ABT-737, can modulate the cellular calcium homeostasis in platelets. Further studies will be needed to address the functional consequences of this calcium response in vivo and its contribution to the thrombocytopenia observed on ABT-263 administration.

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sis, disrupts cellular calcium homeostasis, and prevents platelet activation.

To the editor:

Doubts concerning functional endothelial nitric oxide synthase in human erythrocytes

Nitric oxide (NO) is involved in the modulation of multiple physiologic functions. NO is produced from L-Arg by the catalytic action of NO synthase (NOS; EC 1.14.13.39). Erythrocytes have been reported to express NOS, an eNOS isoform. However, findings on a functional erythrocytic eNOS (eeNOS) in humans are contradictory. Discrepancies may be because of different experimental conditions and methodological shortcomings. In consideration of the paradoxical occurrence of eeNOS in red blood cells (RBCs), which are mainly responsible for NO inactivation, we attempted to measure NOS activity in RBCs freshly collected from normal healthy humans by a fully validated, highly sensitive and specific gas chromatography-mass spectrometry (GC-MS) assay. Approval from the local Ethics Committee of the Hannover Medical School was obtained. Blood was drawn from the antecubital vein of healthy male and female volunteers using EDTA monovettes and processed immediately. RBCs were separated by centrifugation (800g, 4°C, 5 minutes) and used either unwashed or after repeated wash with physiologic saline. Unwashed RBCs were lyzed by freezing (30 minutes, −80°C) followed by slow defrosting in an ice bath and by rapid vortex-mixing (1 minute) with ice-cold distilled water (1:1, vol/vol).

Both in whole blood and in washed RBCs, externally added L-[guanidine-15N2]-arginine (L-[15N2]-Arg; Figure 1A) but not 15NO-derived [15N]nitrite and [15N]nitrate (not shown) was detected in RBCs cytosol at concentrations comparable with erythrocytic L-Arg concentrations. In washed RBCs isolated from blood of a healthy female volunteer, the peak area ratio (PAR) of m/z 47 ([15N]nitrite) to m/z 46 ([14N]nitrite) and the PAR of m/z 63 ([15N]nitrate) to m/z 62 ([14N]nitrate) measured in the RBCs cytosol did not differ between untreated and L-[15N2]-Arg-treated RBCs (Figure 1B). These findings suggest no formation of 15NO from L-[15N2]-Arg by native RBCs.

We did not find [15N]nitrite and [15N]nitrate above baseline levels in lyzed RBCs from freshly obtained blood of 5 healthy volunteers on incubation with L-[15N2]-Arg (Figure 1C). In contrast, external addition of a recombinant human eNOS (heNOS) resulted in formation of [15N]nitrate indicating functional heNOS activity in lyzed RBCs. Addition of NADPH to lyzed RBCs did not further increase heNOS activity suggesting that sufficient endogenous NADPH is present in the hemolysate (Figure 1C). heNOS activity in buffer was found not to differ for H2B concentrations between 100 and 2500nM. That heNOS activity was measurable in lyzed RBCs suggest that H2B is present in lyzed and native RBCs at concentrations high enough to ensure NOS activity. In addition, we found that glutathione reductase (GR), which shares with NOS the cofactors NADPH and FAD, was active in lyzed RBCs (Figure 1D). This finding suggests that other pathways are intact and functional in the lyzed RBCs used in the present study.

We used a sophisticated GC-MS assay to measure NOS activity in human RBCs. Our results suggest that human RBCs either...
contain inactive eeNOS or eeNOS activity is very low. The physiologic function of eeNOS in human erythrocytes is elusive and remains to be established.

Figure 1. Uptake of L-[15N2]-Arg in RBCs and assessment of NO synthase and glutathione reductase activity in intact and lyzed human RBCs. Enrichment of L-[15N2]-Arg (A), [15N]Nitrite, [15N]Nitrate and L-[15N2]-Arg (B) in RBCs cytosol after incubation of L-[15N2]-Arg with whole blood and washed RBCs of 2 healthy volunteers (A,B); NOS activity in lyzed human RBCs in the absence and in the presence of externally added heNOS (C); glutathione reductase (GR) activity in lyzed human RBCs (D).

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