activation, and challenges the concept that NO/cGMP solely plays an inhibitory role in platelets. Gambaryan et al claim that they “have never obtained evidence for any ‘stimulatory’ role of sGC in platelets” and conclude that “…the NO/sGC/cGMP/PKG pathway plays exclusively inhibitory roles in platelets.” With due respect, we wish to point out that inability of someone or some groups to see something does not prove its nonexistence. Thus, Gambaryan et al are logically incorrect in making the conclusion. Furthermore, there has been extensive data support from the Du laboratory, the Li laboratory, and others that the NO/sGC/cGMP/PKG pathway plays a stimulatory role in platelets (in addition to its inhibitory role), and there is a PI3K/Akt/eNOS/sGC/cGMP/PKG signaling pathway that stimulates platelet granule secretion.2 Nevertheless, we are happy to discuss with Gambaryan et al to resolve the possible technical issues that prevent them from observing the stimulatory role of cGMP.

There is a labeling error in Figure 2A in our Blood article.2 The unit of cGMP concentration should be “fmol,” but was inadvertently labeled as “pmol.” We apologize for this error. However, there is an increase in cGMP levels in platelets stimulated with thrombin, which is consistent with previous results,5,6 and with the well-established concept that platelet activation leads to cGMP elevation.11 Previously we reported that VWF induces cGMP elevation in platelets.2 Gambaryan et al disputed our data and reported that they could not stably detect platelet cGMP elevation.12 However, with some apparent adjustment in techniques, Gambaryan et al have recently been able to show the elevation of cGMP in response to VWF.13 Similarly, we believe that detection of thrombin-induced cGMP elevation is a technical issue associated with their assay sensitivity.

VASP (vasodilator-stimulated phosphoprotein) phosphorylation in platelets is not a reliable indicator of platelet cGMP levels. VASP phosphorylation in human platelets can be mediated by cAMP-dependent protein kinase.14

It is true that Marcondes et al reported data supporting cGMP-independent inhibition of platelets by NO donors in 2006.15 However, this conclusion was disputed by Friebe, a coauthor of the Gambaryan letter, who reported that inhibition of platelets by NO donors is solely dependent on sGC.16 Our results show that sGC knockout did not affect platelet inhibition by high concentrations of NO donors. Therefore, we feel that it is necessary to report our findings, which support the conclusion of Marcondes et al. It is good to know that authors of this letter including Dr Friebe now agree that high concentrations of NO donors can inhibit platelets via sGC-independent mechanisms.

Zhenyu Li
Department of Medicine, University of Kentucky, Lexington, KY

Xiaoping Du
Department of Pharmacology, University of Illinois at Chicago, Chicago, IL

Contribution: Z.L. and D.X. wrote the letter.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Zhenyu Li, Saha Cardiovascular Center, 741 S Limestone St, BBSRB, Rm B251, Lexington, KY 40536-0200; e-mail: zhenyuli08@uky.edu; and Xiaoping Du, Department of Pharmacology, University of Illinois College of Medicine, 835 W Wolcott Ave, Chicago, IL 60612; e-mail: xdu@uic.edu.

References

These mechanisms include inhibition of thromboxane A2 (TxA2) synthesis by platelet cyclooxygenase-1 (COX-1), blockade of the platelet TxA2 receptor, and S-nitrosation of cysteine moieties on the platelet surface.3 SNP is a potent inhibitor of ADP-induced (2μM) human platelet aggregation in platelet-rich plasma (PRP) by cGMP-dependent and cGMP-independent mechanisms.3 These effects were seen at an SNP concentration of only 1μM, which is 500 times lower than the SNP concentration used by Zhang et al1 in mice. At very high concentrations, experimental NO/NO+ donors may exert concentration-dependent, diametrically different effects on platelet biochemistry.2 Our observations indicate that NO/NO+ donors can both decrease and increase COX-1 activity (Figure 1A-B).

NO is endogenously produced in various types of cells by constitutive and inducible NO synthase (NOS) isoforms and plays multiple physiologic roles.6,7 Blood platelets and red blood cells are considered to express NOS isoforms, but there are doubts about functional erythrocytic8 and platelet4,9 NOS. Using a sophisticated gas chromatography–mass spectrometry method, we did not detect any NOS activity in human platelets (Figure 1C). Addition of functional recombinant human endothelial NOS to human PRP resulted in NO formation and platelet aggregation inhibition (Figure 1C; supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

In summary, in human blood platelets and erythrocytes, expression and functionality of NO synthase are in dispute. Extra-platelet NO/NO+ species including endothelium-derived NO and NO/NO+-releasing drugs are potent inhibitors of platelet aggregation. They are unlikely to modulate platelet function by influencing platelet L-arginine/NO pathway, but they may modulate platelet COX-1. Effects of NO/NO+ donors on platelet function observed experimentally at therapeutically irrelevently high drug concentrations are rather artifactual and misleading. Extension of observations on platelet function from mice to humans should be treated with caution.

Dimitrios Tsikas
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

Markus Flentje
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

Jonas Niemann
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

Anke Böhmer
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

Dirk O. Stichtenoth
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

References

Response

Inhibition of platelet activation by NO involves both cGMP-dependent and -independent mechanisms

We are grateful for the opportunity to reply to the letter from Tsikas et al in regard to our recent publication in Blood. 2 Tsikas et al write that “Zhang and colleagues stated in their recent article that it was unknown until their study whether NO donors inhibit platelet function by cGMP-dependent and cGMP-independent mechanisms.”3-5 The truth is that we discussed previous findings by citing important literature that NO inhibits platelet function by both cGMP-dependent (references 11, 12, and 16 in Zhang et al)6 and -independent mechanisms (references 38, 39, 40, 43, and 45 in Zhang et al).6 Although previous reports suggested cGMP-independent mechanisms involved in NO-mediated platelet inhibition, this conclusion was recently disputed by the Friebe group, which reported that inhibition of platelets by NO donors is solely dependent on soluble guanylyl cyclase (sGC) using sGC whole-body knockout mice.3-5 Therefore, whether cGMP-independent mechanisms are involved in NO-mediated inhibition of platelets is unclear. I agree that it would be more accurate to say “whether the inhibitory effect of NO on platelet activation involves these sGC/cGMP-independent mechanisms is unclear,” instead of saying “whether the inhibitory effect of NO on platelet activation involves these sGC/cGMP-independent mechanisms is not known.” Our results show that NO donors at high concentrations inhibited platelet function from sGC conditional knockout mice, which support the conclusion that cGMP-independent mechanisms are involved in NO-mediated platelet inhibition and are also consistent with the previous reports from the authors of this letter.

The data in the letter that showed NO/NO donors can both decrease and increase COX-1 activity in platelets are not contradictory to our results but support our findings that NO/cGMP plays a biphasic role in platelet activation.6,4 Tsikas et al show in their letter that they failed to detect NOS activity in platelets using a sophisticated gas chromatography–mass spectrometry method.1 In our paper we reported that sGC is a required agonist-induced platelet activation and that exogenous NO donors inhibit platelet aggregation and secretion through sGC-dependent and -independent mechanisms.7 Thus our study does not involve whether or not platelets express NO. However, data shown in the letter are contradictory not only to many previous reports from different groups,8-14 but also to a report from the authors of the letter that L-arginine inhibits platelet aggregation and thromboxane A2 formation through NOS dependent mechanisms (reference 2 in Tsikas et al).1

References


Zhenyu Li
Department of Medicine, University of Kentucky, Lexington, KY

Contribution: Z.L. wrote the letter.

Conflict-of-interest disclosure: The author declares no competing financial interests.

Correspondence: Zhenyu Li, Saha Cardiovascular Center, 741 S Limestone St, BBSRB, Rm B251, Lexington, KY 40536-0200; e-mail: zhenyuli08@uky.edu.
Extra-platelet NO and NO\(^{\bullet}\)-containing drugs are potent inhibitors of platelet aggregation in humans by cGMP-dependent and cGMP-independent mechanisms

Dimitrios Tsikas, Markus Flentje, Jonas Niemann, Anke Böhmer and Dirk O. Stichtenoth