In their discussion, Zhang et al state that the 2 major findings in their recent study are: first, that sGC plays a stimulatory role in platelet activation.1 This does not agree with several other studies as mentioned previously. Second, they reported that millimolar concentrations of SNP can mediate sGC-platelet inhibition. This was already described for human platelets by Marcondes et al in 2006,6 which was even cited by Zhang et al1 (reference 43 in Zhang et al), but for other reasons.

In summary, in our experiments we show that thrombin neither increases cGMP nor the subsequent cGMP-dependent VASP phosphorylation in platelets. Our data and those of many other groups do not support a “sGC platelet stimulatory concept.” In contrast, our present data and that of many other authors confirm the concept that the NO/sGC/cGMP/PKG pathway plays exclusively inhibitory roles in platelets.

Response

Yes, cGMP plays a stimulatory role in platelet activation

We are grateful for the opportunity to reply to Gambaryan et al1 who dispute conclusions of our recent publication.2 In our study, we have shown that soluble guanylyl cyclase (sGC) plays biphasic roles in platelet activation, a stimulatory role during platelet activation induced by low-dose platelet agonists and an inhibitory role when stimulated with high or pharmacologic concentrations of NO donors. Our finding extends our previous discoveries that the NO/cGMP/PKG signaling pathway plays biphasic roles in platelet activation, a stimulatory role during platelet activation induced by low-dose platelet agonists and an inhibitory role when stimulated with high or pharmacologic concentrations of NO donors. Our finding extends our previous discoveries that the NO/cGMP/PKG signaling pathway plays biphasic roles in platelets.
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. 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. 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, and with the well-established concept that platelet activation leads to cGMP elevation. Previously we reported that VWF induces cGMP elevation in platelets. Gambaryan et al disputed our data and reported that they could not stably detect platelet cGMP elevation. However, with some apparent adjustment in techniques, Gambaryan et al have recently been able to show the elevation of cGMP in response to VWF. 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. It is true that Marcondes et al reported data supporting cGMP-independent inhibition of platelets by NO donors in 2006. 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. 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.

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References

To the editor:

Extra-platelet NO and NO+ containing drugs are potent inhibitors of platelet aggregation in humans by cGMP-dependent and cGMP-independent mechanisms

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. Yet we and others reported several years ago that S-nitrosocysteine (CSNO) and sodium nitroprusside (SNP; Na2[Fe(CN)6NO]) inhibit platelet function by cGMP-dependent and cGMP-independent mechanisms. CSNO is a potent NO and NO+ donor, a crucially important extra- and intra-cellular S-nitrating species, and a potent inhibitor (IC50, 100nM) of collagen-induced (1 μg/mL) aggregation of washed human platelets by different cGMP-independent mechanisms.
Response: yes, cGMP plays a stimulatory role in platelet activation

Zhenyu Li and Xiaoping Du