Effects of the sGC Stimulator BAY 41-2272 Are Not Mediated by Phosphodiesterase 5 Inhibition

To the Editor:

A major conclusion of the recent publication of Mullershausen et al. is that “the physiological effects of BAY 41-2272 . . . are due to the synergism of sensitization of NO-sensitive GC [guanylate cyclase] and inhibition of PDE5.” This conclusion is based on the authors’ finding that BAY 41-2272 stimulates sGC and inhibits human PDE5A1 at the same half-maximal concentration of 3 μM. These observations are inconsistent with our own observations as well as results generated by others.

We have hypothesized that the only significant activity of BAY 41-2272 is the NO-independent activation of NO-sensitive GC. In our laboratory, as little as 0.001 μM BAY 41-2272 stimulates the highly purified recombinant sGC, and maximal stimulation is achieved by 1 μM. Moreover, BAY 41-2272 activates sGC in a stably sGC-overexpressing CHO cell line and in a cGMP reporter cell line with EC50s of 0.09 μM and 0.17 μM, respectively. Even in tissues, IC50s for BAY 41-2272 have been reported by Cellek’s group several times that are 6- to 20-fold lower than the 3-μM range observed by Mullershausen; these include anococcygeus muscle from control and in a cGMP reporter cell line with EC50 of 0.09 μM and 0.17 μM.

On the other hand, we find that BAY 41-2272 fails to significantly inhibit highly purified recombinant human PDE5 expressed in a baculovirus system at concentrations up to 10 μM (confirmed at MDS Pharma Services), whereas the PDE5 inhibitors sildenafil and our vardenafil show IC50 values of 0.007 and 0.0007 μM, respectively. Moreover, BAY 41-2272 also does not inhibit other cGMP-specific/metabolizing PDEs, such as PDE-1, -2 and -9.

Taking all these points into account, we believe that Mullershausen overestimated the potency of BAY 41-2272 on PDE5 and underestimated its potency on sGC.

In an effort to validate their hypothesis in a cellular system, Mullershausen next demonstrated that BAY 41-2272 at 100 μM elevates platelet cGMP in the presence of an NO donor and that a mixture of sildenafil and EHNA (which inhibits both PDE2 and PDE5) also elevates cGMP under these conditions. Because these results would be anticipated without ascribing PDE5-inhibitory activity to BAY 41-2272, they do not test its hypothetical synergistic mechanism. Furthermore, it is unclear to us why BAY 41-2272 was used at 100 μM when we have demonstrated its antiaggregatory effect with an IC50 of 0.04 μM, and Hobbs and Moncada have shown its antiplatelet effect with concentrations between 0.01 μM and 0.3 μM.

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Response

We are grateful for the opportunity to reply to the letter from Bischoff and Stasch about our publication. They claim that we underestimated the potency of BAY41-2272 on guanyl cyclase (GC) and overestimate its inhibitory potency on phosphodiesterase type 5 (PDE5).

BAY41-2272 sensitizes GC toward nitric oxide (NO) as it shifts the EC50 for NO by 1.5 orders of magnitude to the left. BAY41-2272 alone activates the enzyme NO-independently 30-fold, whereas maximal NO stimulation is 200-fold. We measured an EC50 of 0.3 μM for BAY41-2272 in the presence of NO (100 nmol/L DEA-NO) and 3 μM in the absence of NO. Considering the greater potency of BAY41-2272 in the presence of NO and its tremendous effect on enzyme activity at low (physiological) NO concentrations, we are surprised that Stasch and Bischoff hypothesized that “. . .the only significant effect of BAY41-2272 is the NO-independent activation of GC.”

Unfortunately, in the original publication, the authors did not provide any EC50 values for BAY41-2272, and the double logarithmic plot hampers their estimation. The statement in their letter that “. . . as little as 0.001 μM BAY41-2272 stimulates the highly purified recombinant sGC . . .” is misleading considering the marginal activation (2-fold versus maximally 400-fold). EC50 values for BAY41-2272 of 0.5 μM for NO-independent activation and 0.1 μM in the presence of NO (100 nmol/L DEA-NO) have been published with Stasch as coauthor. These values are in a reasonable agreement with our data.

Bischoff and Stasch claim that lower BAY41-2272 concentrations induce physiological responses. This is not surprising as NO also elicits physiological effects at concentrations by far lower than those that elicit measurable cGMP elevations.

Stasch and Bischoff state that “. . . BAY41-2272 fails to inhibit . . . PDE5 . . . at concentrations up to 10 μM.” We observed that inhibition of PDE5 by BAY41-2272 critically depends on the substrate concentration indicating competition. With high substrate (>10 μM cGMP), BAY41-2272 (10 μM) will not inhibit PDE5, whereas at low substrate (0.1 μM cGMP), BAY41-2272 effectively inhibits PDE5 (50% inhibition at 3 μM BAY41-2272, Figure 1C). Unfortunately, Stasch never provided experimental details.

In platelets, maximal NO elicits a transient elevation of cGMP (300 pmol cGMP/106 platelets), which is reversed within 40 s by PDE5 activation. With PDE inhibitors, cGMP accumulated to a plateau of 3000 pmol/106, revealing the importance of PDE activity for the transient response. BAY41-2272 and maximal NO caused cGMP accumulation to a plateau of 2000 pmol/106.

Bischoff and Stasch state that the observed response “. . . would be anticipated without ascribing PDE5-inhibitory activity to BAY41-2272 . . . .” Our results argue against this assumption. Using purified GC, similar activities are induced either by maximal NO (100 μM DEA-NO) or subthreshold NO with BAY41-2272 (0.1 μM DEA-NO, 100 μM BAY41-2272; Figure 1A). However, the respective cGMP responses in platelets differed substantially (Figure 2). A transient cGMP response was elicited by maximal NO alone (100 μM GSNO), whereas with subthreshold NO and BAY41-2272 (3 μM)
GSNO, 100 μmol/L BAY41-2272), cGMP accumulated to a plateau 3-fold higher than the transient response.

In sum, the effects of BAY41-2272 on platelet cGMP cannot be solely explained by activation of GC but by the combined action on GC and PDE5 consistent with our in vitro results.

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