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 μmol/L. 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 μmol/L BAY 41-2272 stimulates the highly purified recombinant sGC, and maximal stimulation is achieved by 1 μmol/L. Moreover, BAY 41-2272 activates sGC in a stably sGC-overexpressing CHO cell line and in a cGMP reporter cell line with EC50 of 0.09 μmol/L and 0.17 μmol/L, respectively. Even in tissues, IC50 for BAY 41-2272 have been reported by Cellek’s group several times that are 6- to 20-fold lower than the 3-μmol/L range observed by Mullershausen; these include anococcygeus muscle from control and in a cGMP reporter cell line with EC50 of 0.09 μmol/L and 0.17 μmol/L.

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 μmol/L (confirmed at MDS Pharma Services), whereas the PDE5 inhibitors sildenafil and our vardenafil show IC50 values of 1; these include anococcygeus muscle from control and in a cGMP reporter cell line with EC50 of 0.09 μmol/L and 0.17 μmol/L. 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 μmol/L elevates platelet cGMP in the presence of an NO donor and that a mixture of sildenafil and EHNQ (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 μmol/L when we have demonstrated its antiaggregatory effect with an IC50 of 0.04 μmol/L, and Hobbs and Moncada have shown its antiplatelet effect with concentrations between 0.01 μmol/L and 0.3 μmol/L.

Erwin Bischoff, PhD
Johannes-Peter Stasch, PhD, PharmD
Cardiovascular Research
Bayer HealthCare
Wuppertal, Germany


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 BAY 41-2272 on guanylyl cyclase (GC) and overestimate its inhibitory potency on phosphodiesterase type 5 (PDE5).

BAY 41-2272 sensitizes GC toward nitric oxide (NO) as it shifts the EC50 for NO by 1.5 orders of magnitude to the left. BAY 41-2272 alone activates the enzyme NO-independently 30-fold, whereas maximal NO stimulation is 200-fold. We measured an EC50 of 0.3 μmol/L for BAY 41-2272 in the presence of NO (100 nmol/L DEA-NO) and 3 μmol/L in the absence of NO. Considering the greater potency of BAY 41-2272 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 BAY 41-2272 is the NO-independent activation of GC.”

Unfortunately, in the original publication the authors did not provide any EC50 values for BAY 41-2272, and the double logarithmic plot hampers their estimation. The statement in their letter that “. . . as little as 0.001 μmol/L BAY 41-2272 stimulates the highly purified recombinant sGC . . .” is misleading considering the marginal activation (2-fold versus maximally 400-fold). EC50 values for BAY 41-2272 of 0.5 μmol/L for NO-independent activation and 0.1 μmol/L 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 BAY 41-2272 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 “. . . BAY 41-2272 fails to inhibit . . . PDE5 . . . at concentrations up to 10 μmol/L.” We observed that inhibition of PDE5 by BAY 41-2272 critically depends on the substrate concentration indicating competition. With high substrate (>10 μmol/L cGMP), BAY 41-2272 (10 μmol/L) will not inhibit PDE5, whereas at low substrate (0.1 μmol/L cGMP), BAY 41-2272 effectively inhibits PDE5 (50% inhibition at 3 μmol/L BAY 41-2272, 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. BAY 41-2272 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 BAY 41-2272 . . . . . .” Our results argue against this assumption. Using purified GC, similar activities are induced either by maximal NO (100 μmol/L DEA-NO) or subthreshold NO with BAY 41-2272 (0.1 μmol/L DEA-NO, 100 μmol/L BAY 41-2272; Figure 1A). However, the respective cGMP responses in platelets differed substantially (Figure 2).
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.

Florian Mullershausen, PhD
Michael Russwurm, MD
Andreas Friebe, PhD
Doris Koesling, MD
Institut für Pharmakologie und Toxikologie
Medizinische Fakultät
Ruhr-Universität Bochum
Bochum, Germany
doris.koesling@ruhr-uni-bochum.de

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Erwin Bischoff and Johannes-Peter Stasch

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