Soluble Guanylate Cyclase

Not a Dull Enzyme

Guido Boerrigter, MD; John C. Burnett, Jr, MD

Arthur Kornberg, who shared with Severo Ochoa the 1959 Nobel Prize for Physiology or Medicine, stated in his autobiography that he had never met a dull enzyme.\(^1\) We strongly believe that soluble guanylate cyclase (sGC; E.C.4.6.1.2) is no exception to this.

Nitric oxide (NO) plays an important physiological role as a signaling molecule as well as a cytotoxic agent. It is produced by 3 distinct NO synthases, specifically endothelial, neuronal, and inducible NO synthase. In the vasculature, endothelial NO synthase plays an important role in vascular homeostasis because NO generated in endothelial cell promotes vascular smooth muscle cell relaxation and inhibits platelet aggregation. Importantly, bioavailability of NO can be reduced in cardiovascular diseases, a condition which has been termed “endothelial dysfunction.” This phenomenon provides a rationale for therapeutic intervention.

The primary target of NO is sGC, which is a heterodimeric enzyme consisting of an \(\alpha\)- and \(\beta\)-subunit and a prosthetic heme group, which is located in the \(\beta\)-subunit. The heme group contains a ferrous iron atom (Fe\(^{2+}\)). Binding of NO to this iron changes the conformation of the enzyme and leads to a >100-fold increase in catalytic rate (ie, the conversion of guanosine triphosphate to the second messenger cyclic guanosine monophosphate [cGMP]). The NO-induced cGMP signal modulates intracellularly the activity of several effector molecules: cGMP-dependent protein kinases, cGMP-regulated phosphodiesterases, and cGMP-gated ion channels. Of note, both heme iron oxidation (Fe\(^{3+}\)) and heme removal render sGC unresponsive to NO (Figure). These 2 conditions could therefore provide a mechanism for “vascular smooth muscle cell dysfunction” in cardiovascular disease states, which are often characterized by marked vasoconstriction either systemically or in specific organs.

Therapeutic administration of exogenous NO mimetics such as organic nitrates has been used for more than a century.\(^2\) However, nitrates have potential disadvantages including the development of tolerance, cGMP-independent actions, protein nitrosation, and oxidative stress.\(^3\) Given this background, great interest met the report by Ko et al in 1994 that the benzylindazole YC-1 was able to activate sGC directly (ie, in an NO-independent manner).\(^4\) Using a structure-driven approach with YC-1 as a lead compound and a high-throughput screening method, Stasch et al identified 2 different classes of NO-independent sGC stimulators, one of which was heme dependent whereas the other was heme independent.\(^5,6\) Importantly, neither class was associated with the development of tolerance after long-term administration, and both classes were effective in nitrate-tolerant vessels.

The first orally available sGC stimulator to be introduced in 2001 was BAY 41–272, the photolabeling of which suggested that it binds to a regulatory site on the \(\alpha\) subunit of sGC.\(^5\) BAY 41–2272 activation of sGC is synergistic with NO, and a related compound, BAY 41–8543, prolonged the half-life of the nitrosyl-heme complex.\(^7\) BAY 41–2272 decreased blood pressure in rats, and it reduced systemic and renal vascular resistance and increased cardiac output and renal blood flow in canine experimental heart failure (HF).\(^5,8\) However, BAY 41–2272 did not reduce right atrial pressure in experimental HF, which would likely be desirable for a HF drug.\(^9\) Further research led to the development of riociguat (BAY 63–2521), a compound with improved oral pharmacokinetics that is currently in clinical development for the treatment of pulmonary arterial hypertension.\(^9\) Importantly, these NO-independent but heme-dependent sGC stimulators,

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**Figure.** Schematic illustrating 3 different forms of soluble guanylate cyclase and their respective responsiveness to nitrovasodilators, sGC stimulators (eg, BAY 41–2272), and NO and nitrovasodilators only stimulate sGC, which contains the heme moiety with a ferrous iron (Fe\(^{2+}\)); furthermore, NO and nitrovasodilators have cGMP-independent actions. BAY 41–2272 also activates only the NO-sensitive sGC but without the cGMP-independent actions of NO and nitrovasodilators. Cinaciguat activates heme-free sGC, which is insensitive to NO. The question marks indicate that little is known about the transition between the different forms, their prevalence in health and disease, and their potential restoration to the reduced NO-sensitive form.
like NO, do not activate sGC if the heme iron is oxidized or if the heme is absent.

Cinaciguat, also known as BAY 58–2667, is an NO-independent and heme-independent sGC activator first described in 2002.6 Activation of sGC by cinaciguat was not synergistic with NO but only additive, and cinaciguat did not affect the half-life of the nitrosyl-heme complex.7 It should be noted here that nonnitrosylated heme can be considered an inhibitor of sGC activity whereas nitrosylated heme strongly activates the enzyme.10 Oxidation of the heme iron not only makes sGC unresponsive to NO, but it also facilitates removal of the heme, which promotes degradation of the enzyme.11 Cinaciguat seems to exclusively activate the heme-free sGC by binding in the heme pocket and thus changing the enzyme conformation, which not only activates sGC but also inhibits its degradation.11–13 Given that cinaciguat has demonstrated biological actions in health and disease, we have evidence that a significant pool of heme-free sGC exists in both physiological and pathophysiological conditions.6,13,14–16

Importantly, Stasch et al demonstrated that in isolated vessels from different vascular disease models, responsiveness to conventional NO donors decreased whereas sensitivity to cinaciguat increased, consistent with a reduction in NO-responsive nonoxidized sGC and an increase in heme-free sGC. These findings demonstrate that, in addition to being a potential new drug, cinaciguat is also an interesting pharmacological tool. In canine experimental HF, cinaciguat had hemodynamic actions that were qualitatively identical to those seen with nitroglycerin, which included systemic and renal vasodilation, increase in cardiac output and renal blood flow, maintenance of renal function, and reduction in right atrial pressure.8,14 The difference in the venous versus arterial dilation seen with cinaciguat and BAY 41–2272 may be due to local metabolism, a higher prevalence of heme-free sGC in the venous circulation, and a higher endogenous NO availability in the arterial circulation.

In the current issue of Circulation, Lapp et al provide the first data on the use of cinaciguat in human HF.16 Cinaciguat infusion showed promising cardiac unloading actions, with reductions in cardiac filling pressures and systemic and pulmonary vascular resistance and an increase in cardiac output. As discussed by the authors, this was a proof-of-concept study without a control group and therefore interpretation of symptomatic improvement is difficult. From a drug development perspective, the study by Lapp et al is another encouraging step forward, and the logical next step, a placebo-controlled, randomized, double blind, Phase IIb study, has already been started (ClinicalTrials.gov Identifier: NCT00559650).

It should be noted, however, that clinical trials in acute decompensated HF are not necessarily straightforward, which can be illustrated with some recent examples. In the Vasodilator in the Management of Acute CHF (VMAC) trial, B-type natriuretic peptide (ie, Nesiritide) led to a significantly larger reduction in pulmonary capillary wedge pressure at 3 hours than placebo and nitroglycerin.17 However, the dosing, which included an initial bolus, was associated with an increased incidence of hypotension, which likely contributed to a signal for worsened renal outcomes.18 Indeed, the optimal and most meaningful end points for clinical trials in acute decompensated HF are unclear, and it is increasingly acknowledged that a relatively short-term treatment during hospitalization may not be able to affect long-term outcomes. Also, HF is a very heterogenous syndrome and not every patient is likely to benefit from every intervention. Therefore, patient selection for a clinical trial may be crucial. Indeed, the recent Preliminary Study of Relaxin in Acute Heart Failure (pre-RELAX-AHF) that evaluated the vasodilator hormone relaxin specifically targeted patients with mild to moderate renal insufficiency and systolic blood pressure >125 mm Hg (for comparison: an exclusion criterion in the VMAC trial was a systolic blood pressure <90 mm Hg),17,19 The best clinical indication for cinaciguat remains to be established.

In the study by Lapp et al, cinaciguat was given as an intravenous drug in the setting of acute decompensated HF. Of similar interest would be a heme-independent sGC activator with good oral pharmacokinetics for the long-term treatment of patients with HF. Clinical trials in HF with long-term administration of conventional sGC stimulators (ie, nitrates) have been associated with improved outcomes despite their above-mentioned potential shortcomings. For example, the African American Heart Failure Trial (A-HeFT) most recently showed improved survival with a combination of isosorbide dinitrate and hydralazine in self-identified African Americans.20 These findings should be incentive for the pharmaceutical industry to develop orally available heme-independent sGC activators, which would enable us to assess chronic sGC activation in HF or other cardiovascular disease states without cGMP-independent actions or tolerance development. The Table provides an overview of previously reported heme-dependent and heme-independent sGC stimulators.

In summary, there is a truly unmet need for novel, safe, and effective therapies for acute decompensated HF. Advances in molecular pharmacology in the broad field of cGMP activators and modulators have clearly resulted in innovative molecules that are able to bypass impaired NO/sGC/cGMP signaling in models of endothelial–vascular smooth muscle dysfunction. The translational work of Lapp and coworkers should be viewed as a seminal step forward in taking such advances to the bedside. Continuing human trials in HF and other cardiovascular disease syndromes will tell us if we are headed in the right direction.

### Table. NO-Independent Stimulators of Soluble Guanylate Cyclase

<table>
<thead>
<tr>
<th>NO-Independent but Heme-Dependent sGC Stimulators</th>
<th>NO- and Heme-Independent sGC Activators</th>
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<tbody>
<tr>
<td>YC-1</td>
<td>BAY 58–2667 (cinaciguat)</td>
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<tr>
<td>CFM-1571</td>
<td>BAY 60–2770</td>
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<tr>
<td>BAY 41–2272</td>
<td>HMR1766 (ataciguat)</td>
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<tr>
<td>BAY 41–8543</td>
<td>S3448</td>
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<tr>
<td>BAY 63–2521 (riociguat)</td>
<td>A-350619</td>
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<tr>
<td>A-344905</td>
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References
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