

# Cardiorenal and Humoral Properties of a Novel Direct Soluble Guanylate Cyclase Stimulator BAY 41-2272 in Experimental Congestive Heart Failure

Guido Boerrigter, MD; Lisa C. Costello-Boerrigter, MD, PhD; Alessandro Cataliotti, MD; Toshihiro Tsuruda, MD, PhD; Gail J. Harty, VMT; Harald Lapp, MD; Johannes-Peter Stasch, PhD; John C. Burnett, Jr, MD

**Background**—BAY 41-2272 is a recently introduced novel orally available agent that directly stimulates soluble guanylate cyclase (sGC) and sensitizes it to its physiological stimulator, nitric oxide. To date, its therapeutic actions in congestive heart failure (CHF) remain undefined. We characterized the cardiorenal actions of intravenous BAY 41-2272 in a canine model of CHF and compared it to nitroglycerin (NTG).

**Methods and Results**—CHF was induced by rapid ventricular pacing for 10 days. Cardiorenal and humoral function were assessed at baseline and with administration of 2 doses of BAY 41-2272 (2 and 10  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; n=8) or NTG (1 and 5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; n=6). Administration of 10  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  BAY 41-2272 reduced mean arterial pressure (113±8 to 94±6 mm Hg;  $P<0.05$ ), pulmonary artery pressure (29±2 to 25±2 mm Hg;  $P<0.05$ ), and pulmonary capillary wedge pressure (25±2 to 20±2 mm Hg;  $P<0.05$ ). Cardiac output (2.1±0.2 to 2.3±0.2 L/min;  $P<0.05$ ) and renal blood flow (131±17 to 162±18 mL/min;  $P<0.05$ ) increased. Glomerular filtration rate was maintained. There were no changes in plasma renin activity, angiotensin II, or aldosterone. NTG mediated similar hemodynamic changes and additionally decreased right atrial pressure and pulmonary vascular resistance.

**Conclusion**—The new sGC stimulator BAY 41-2272 potently unloaded the heart, increased cardiac output, and preserved glomerular filtration rate without activation of the renin-angiotensin-aldosterone system in experimental CHF. These beneficial properties make direct sGC stimulation with BAY 41-2272 a promising new strategy for the treatment of cardiovascular diseases such as CHF. (*Circulation*. 2003;107:686-689.)

**Key Words:** enzymes ■ nitric oxide ■ heart failure ■ pharmacology

Congestive heart failure (CHF) is a common disease with increasing prevalence. Despite advances in medical therapy, it continues to be associated with high morbidity and mortality. Hence, there is a need for agents that complement or go beyond conventional therapy.

Recently, a new therapeutic principle has been introduced with BAY 41-2272 (Figure 1).<sup>1</sup> This orally available molecule not only directly stimulates the heterodimeric heme-protein soluble guanylate cyclase (sGC), but also it increases sGC's sensitivity to its natural stimulator, nitric oxide (NO). This results in an increased intracellular production of the second messenger cyclic guanosine monophosphate (cGMP). Cardiovascular properties of cGMP include vascular smooth muscle relaxation and inhibition of platelet aggregation.<sup>2</sup> Conventional organic nitrates activate sGC indirectly after bioactivation. However, their efficacy is limited by the development of tolerance with chronic administration, which has recently been linked to downregulation of mitochondrial

aldehyde dehydrogenase.<sup>3</sup> In contrast, sGC stimulation with BAY 41-2272 is devoid of tolerance development, as has been demonstrated in a rat model of genetic hypertension.<sup>1</sup> Furthermore, oral administration of BAY 41-2272 lowered mean arterial pressure in spontaneously hypertensive rats, showed antiplatelet activity, and enhanced survival in a low-NO rat model of hypertension.<sup>1</sup>

This study was designed to characterize for the first time the acute cardiorenal actions of the innovative molecule BAY 41-2272 in a large animal model of severe CHF<sup>4</sup> and to compare it with nitroglycerin, a commonly used vasodilator.

## Methods

The current study was performed in male mongrel dogs (weight 20 to 28 kg) in accordance with the Animal Welfare Act and with approval of the Mayo Clinic Animal Care and Use Committee.

Severe CHF was induced by rapid ventricular pacing at 240 beats per minute as described previously.<sup>4</sup> After 10 days of pacing, dogs were anesthetized with pentobarbital, intubated, and ventilated with

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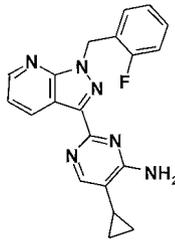
From the Cardiorenal Research Laboratory, Mayo Clinic and Foundation, Rochester, Minn (G.B., L.C.C.-B., A.C., T.T., G.J.H., J.C.B.); and the Department of Cardiology, Wuppertal Heart Center (H.L.), and Pharma Research Center, Bayer AG (J.-P.S.), Wuppertal, Germany.

Correspondence to Guido Boerrigter, MD, Cardiorenal Research Laboratory, Guggenheim 915, Mayo Clinic and Foundation, 200 First St SW, Rochester, MN 55905. E-mail boerrigter.guido@mayo.edu

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BAY 41-2272

Figure 1. Structure of BAY 41-2272.

5 L/min supplemental oxygen. A flow-directed balloon-tipped thermodilution catheter was inserted via the right external jugular vein for hemodynamic measurements. The femoral vein was cannulated for continuous infusions and the femoral artery was cannulated for mean arterial pressure measurements and blood sampling. Pressures were recorded and analyzed digitally (Sonometrics Corporation). Via a left lateral flank incision, the ureter was cannulated for urine sampling and the renal artery was equipped with a flow probe (Carolina Medical Electronics). Cardiac output was measured by thermodilution (Cardiac output computer model 9510-A, American Edwards Laboratories).

One group of dogs received 2 doses of BAY 41-2272 (2 and 10  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; n=8), whereas the other received 2 doses of nitroglycerin (NTG; 1 and 5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; n=6). Doses were chosen in separate dose-finding studies.

The study protocol started with the administration of a weight-adjusted inulin bolus. Continuous inulin and saline infusions at a rate of 1 mL/min each were started. After 60 minutes of equilibrium, a baseline clearance was done. All clearances lasted 30 minutes and consisted of urine collection, blood sampling, and hemodynamic measurements. After the baseline clearance, the saline infusion was

replaced by the lower dose of the study drug (infusion rate 1 mL/min). After a lead-in period of 15 minutes, a 30-minute clearance was done. Thereafter, the higher dose of the study drug was administered in the same manner.

### Analysis of Electrolytes and Neurohormones

Electrolytes were measured by flame photometry (IL943, Instrumentation Laboratory). Glomerular filtration rate (GFR) was assessed by inulin clearance. Plasma renin activity, angiotensin II, and aldosterone were determined by commercially available radioimmunoassays as described previously.<sup>4</sup>

### Statistical Analysis

Values are expressed as mean  $\pm$  SEM. Clearances were compared by repeated-measures ANOVA and Dunnett's test. Statistical significance was accepted at  $P \leq 0.05$ . As the study was not designed to compare the potency of BAY 41-2272 and NTG, the 2 drugs were compared only qualitatively.

## Results

Cardiorenal and humoral function are reported in the Table and Figure 2.

### Cardiovascular Function

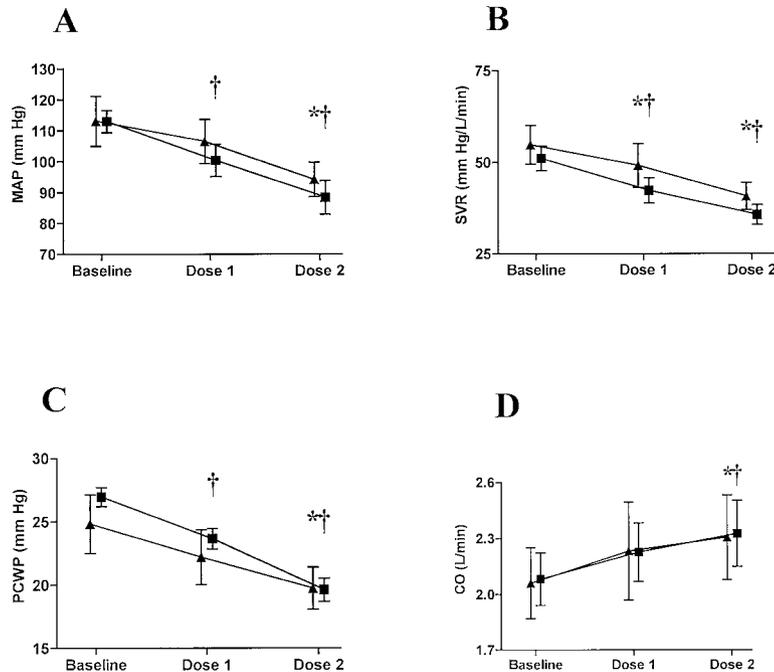
Mean arterial pressure (MAP) decreased with the 10  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  dose of BAY 41-2272 ( $P < 0.001$ ; Figure 2A), whereas systemic vascular resistance (SVR) was reduced with both doses ( $P < 0.05$  and  $P < 0.001$ , respectively; Figure 2B). Right atrial pressure remained unchanged, whereas pulmonary artery pressure ( $P < 0.001$ ) and pulmonary capillary wedge pressure ( $P < 0.001$ ; Figure 2C) decreased with the higher dose of BAY 41-2272. In contrast, pulmonary vascular resistance remained unchanged. Cardiac output (CO) in-

### Cardiorenal and Humoral Function With BAY 41-2272 and Nitroglycerin

	BAY 41-2272			Nitroglycerin		
	Baseline	2 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	Baseline	1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$
<b>Hemodynamics</b>						
Right atrial pressure, mm Hg	3.7 $\pm$ 1.8	3.9 $\pm$ 1.8	3.7 $\pm$ 1.7	8.4 $\pm$ 1.3	7.6 $\pm$ 1.3*	6.4 $\pm$ 1.3*
Pulmonary artery pressure, mm Hg	29.0 $\pm$ 2.4	27.0 $\pm$ 2.4	25.0 $\pm$ 2.2*	33.1 $\pm$ 0.6	28.4 $\pm$ 1.1*	23.6 $\pm$ 1.1*
Pulmonary vascular resistance, mm Hg $\cdot$ L <sup>-1</sup> $\cdot$ min <sup>-1</sup>	2.1 $\pm$ 0.3	2.3 $\pm$ 0.3	2.3 $\pm$ 0.4	3.0 $\pm$ 0.3	2.1 $\pm$ 0.2*	1.7 $\pm$ 0.1*
Renal blood flow, mL/min	131 $\pm$ 17	145 $\pm$ 18*	162 $\pm$ 18*	98 $\pm$ 14	118 $\pm$ 13	145 $\pm$ 18*
Renal vascular resistance, mm Hg $\cdot$ L <sup>-1</sup> $\cdot$ min <sup>-1</sup>	909 $\pm$ 115	790 $\pm$ 122*	596 $\pm$ 57*	1220 $\pm$ 295	823 $\pm$ 125*	603 $\pm$ 88*
<b>Renal function</b>						
Urinary flow, mL/min	0.10 $\pm$ 0.02	0.11 $\pm$ 0.02	0.19 $\pm$ 0.04*	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.09 $\pm$ 0.01
Urinary sodium excretion, $\mu\text{Eq}/\text{min}$	1.5 $\pm$ 0.4	3.0 $\pm$ 0.9	13.4 $\pm$ 10.7	3.3 $\pm$ 0.8	3.1 $\pm$ 0.8	5.0 $\pm$ 1.6
Glomerular filtration rate, mL/min	23 $\pm$ 6	28 $\pm$ 7	26 $\pm$ 5	27 $\pm$ 4	20 $\pm$ 4	24 $\pm$ 6
<b>Humoral function</b>						
Plasma renin activity, ng $\cdot$ mL <sup>-1</sup> $\cdot$ h <sup>-1</sup>	13.3 $\pm$ 1.4	14.0 $\pm$ 1.3	10.6 $\pm$ 1.5	14.1 $\pm$ 2.1	13.1 $\pm$ 2.1	12.3 $\pm$ 2.4
Angiotensin II, pg/mL	588 $\pm$ 303	373 $\pm$ 130	235 $\pm$ 49	684 $\pm$ 336	310 $\pm$ 98	710 $\pm$ 420
Aldosterone, ng/mL	92 $\pm$ 15	99 $\pm$ 15	97 $\pm$ 18	86 $\pm$ 8	90 $\pm$ 12	79 $\pm$ 11

Values are expressed as mean  $\pm$  SEM.

\* $P < 0.05$  compared with baseline.



**Figure 2.** Effect of BAY 41-2272 (▲) and nitroglycerin (■) administration on (A) mean arterial pressure (MAP), (B) systemic vascular resistance (SVR), (C) pulmonary capillary wedge pressure (PCWP), and (D) cardiac output (CO). Dose 1:  $2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of BAY 41-2272 or  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of nitroglycerin. Dose 2:  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of BAY 41-2272 or  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of nitroglycerin. \* $P < 0.05$  compared with baseline for the BAY 41-2272 group; † $P < 0.05$  compared with baseline for the nitroglycerin group.

creased with  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of BAY 41-2272 ( $P < 0.05$ ; Figure 2D). With 2 exceptions, the hemodynamic actions of NTG were qualitatively similar to BAY 41-2272. Unlike BAY 41-2272, however, NTG reduced right atrial pressure ( $P < 0.001$  for both 1 and  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and pulmonary vascular resistance ( $P < 0.05$  and  $P < 0.001$  for 1 and  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively).

### Renal Function

Renal blood flow could not be assessed in 1 study in each group because of technical problems. Renal blood flow increased with BAY 41-2272 ( $P < 0.001$  for both doses), corresponding to a decrease in renal vascular resistance ( $P < 0.001$  for the higher dose). Urine flow slightly increased with the higher dose of BAY 41-2272 ( $P < 0.001$ ). Urinary sodium excretion and GFR remained unchanged. NTG increased renal blood flow and reduced renal vascular resistance ( $P < 0.001$  and  $P < 0.05$  for the  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  dose, respectively) but did not change urine flow, urinary sodium excretion, or GFR (Table).

### Humoral Function

Both BAY 41-2272 and NTG left plasma renin activity, angiotensin II, and aldosterone unchanged.

### Discussion

This study reports for the first time the cardiorenal and humoral actions of direct sGC stimulation and sensitization with BAY 41-2272 in a model of severe CHF. This novel new agent resulted in potent systemic and renal vasodilation, increasing CO while preserving GFR. Several properties make BAY 41-2272 a promising new therapeutic agent that goes beyond current therapeutic agents.

First, BAY 41-2272 acts as an arterial vasodilator, resulting in a reduction of MAP and pulmonary artery pressure and a decrease in SVR and renal vascular resistance. In addition,

direct sGC activation resulted in an increase in CO, like NTG. Further studies are needed to explore if this is primarily due to the decrease in SVR or if there is a positive inotropic effect.

Second, BAY 41-2272 reduced pulmonary capillary wedge pressure in the absence of a decrease in right atrial pressure. This decrease could be due to the afterload reduction but could also be related to a lusitropic action. Indeed, phosphorylation of troponin I by cGMP-dependent protein kinase has been reported to result in improved diastolic distensibility.<sup>5</sup> This unique potential for enhancing diastolic function warrants further investigation.

Third, despite a decrease in MAP, BAY 41-2272 preserved GFR and had a significant though small and not clinically relevant diuretic effect. Preservation of renal function is an equally important property of a CHF drug, as it has been demonstrated that renal insufficiency is a major determinant for heart failure progression and mortality.<sup>6</sup> In addition, despite the vasodilating and diuretic actions, there was no further activation of the renin-angiotensin-aldosterone system (RAAS). This is significant, as long-term activation of this humoral pathway may have potentially deleterious effects.<sup>7</sup>

The current study was not designed to compare BAY 41-2272 and NTG quantitatively. Interestingly, in a qualitative comparison, direct sGC stimulation was not identical with nitrate therapy. Specifically, BAY 41-2272 essentially acted as a pure arterial vasodilator and, unlike NTG, did not decrease right atrial pressure and pulmonary vascular resistance. A possible explanation for the differential actions is that sGC activation by NTG depends on the biotransformation of NTG, which has been reported to be higher on the venous side.<sup>8</sup> Furthermore, cGMP-independent actions of NO may account for the differences. Importantly, in addition to concerns regarding mitochondrial toxicity associated with nitrate bioactivation,<sup>3</sup> there is controversy about some poten-

tially adverse consequences of cGMP-independent actions of NO such as peroxynitrite generation, nitrosation, and apoptosis.<sup>9</sup> As BAY 41-2272 acts solely by enhancing the cGMP pathway via sGC activation, one might speculate that it exerts its beneficial hemodynamic effects without some of the potentially deleterious actions of nitrates or NO. Further studies are clearly needed to explore these differentiating characteristics of such agents.

Our findings suggest a novel mechanism for modulating the NO/sGC pathway in the treatment of cardiorenal disease and for counteracting “endothelial dysfunction.”<sup>10</sup> Thanks to its unique enhancement of the physiological signaling cascade without the development of tolerance,<sup>1</sup> BAY 41-2272 may be beneficial by improving the coupling of the endothelial cell with its target cells.

In conclusion, BAY 41-2272 has potent cardiac unloading actions, increases cardiac output, and preserves renal function without activation of the RAAS in experimental heart failure. Thus, direct sGC stimulation by this novel new class represents a potentially efficacious new strategy in the treatment of cardiovascular diseases, including CHF.

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