ERJ Express. Published on January 7, 2009 as doi: 10.1183/09031936.00039808

First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension

Friedrich Grimminger MD PhD,[*] Gerrit Weimann MD,[†] Reiner Frey MD,[†] Robert Voswinckel MD,[*] Melanie Thamm MD,[*] Desire Bölkow,[*] Norbert Weissmann PhD,[*] Wolfgang Mück PhD,[†] Sigrun Unger MS,[‡] Georg Wensing MD,[†] Ralph T. Schermuly PhD[*] and Hossein-Ardeschir Ghofrani MD[*]

Brief title: Riociguat potential in pulmonary hypertension

[*]Department of Internal Medicine, University Hospital Giessen and Marburg, Giessen, Germany

[†]Clinical Pharmacology, Bayer HealthCare AG, Pharma Research Centre, 42096 Wuppertal, Germany

[‡]Global Biostatistics, Bayer HealthCare AG, Pharma Research Centre, 42096 Wuppertal, Germany

Address for correspondence: Hossein-Ardeschir Ghofrani, MD, Department of Internal Medicine, Medical Clinic II/V, University Hospital Giessen and Marburg GmbH, Klinikstrasse 36, 35392 Giessen, Germany; Tel.: ++49 641 99 42 422; Fax: ++49 641 99 42 419; Email: ardeschir.ghofrani@innere.med.uni-giessen.de

Abstract

Pulmonary hypertension (PH) is associated with impaired production of the vasodilator nitric oxide (NO). Riociguat (BAY 63-2521) acts directly on soluble guanylate cyclase, stimulating the enzyme and increasing sensitivity to low NO levels. This study evaluates riociguat safety, tolerability and efficacy in patients with moderate-to-severe PH (pulmonary arterial hypertension, distal chronic thromboembolic PH or PH with mild-to-moderate interstitial lung disease).

The optimal tolerated dose was identified by incremental dosing in four patients with PH; pharmacodynamic and pharmacokinetic parameters were assessed following single dose administration (2.5 mg or 1 mg) in ten and five patients with PH respectively. All subjects were analysed for safety and tolerability (n=19).

Riociguat had a favourable safety profile at single doses ≤2.5 mg. It significantly improved pulmonary haemodynamic parameters and cardiac index in patients with PH in a dose-dependent manner, to a greater extent than inhaled NO. Although riociguat also had significant systemic effects and showed no pulmonary selectivity, mean systolic blood pressure remained >110 mmHg.

This is the first report describing the use of riociguat in patients with PH. The drug was well-tolerated and superior to NO in efficacy and duration. Riociguat therefore has potential as a novel therapy for PH, and warrants further investigation.

Keywords (From MeSH): clinical trial, phase II; maximum tolerated dose; pharmacokinetics; pulmonary hypertension; soluble guanylyl cyclase; vasodilation

Introduction

Pulmonary arterial hypertension (PAH) defines a group of conditions characterized by increased pulmonary vascular resistance (PVR), leading to reduced right heart function and eventual heart failure [1]. It is a progressive disease with an extremely poor prognosis; if left untreated, median life expectancy following diagnosis is 2.8 years [2]. Although rare in the general population (15–52 cases per million [3,4]), its prevalence increases in association with certain conditions. For example, PAH is diagnosed in approximately 0.5% of patients infected with HIV [5], 8–29% of patients with scleroderma [6,7] and 11–32% of patients with sickle cell disease [8,9].

PAH is caused by pulmonary vasoconstriction with vascular remodelling, formation of plexiform lesions and *in situ* thrombosis, which occur in response to aberrant production of a number of signalling factors. Expression of the vasoconstrictor endothelin is increased, while production of vasodilators such as prostacyclin and nitric oxide (NO) is decreased [10]. In healthy individuals, endothelial cell-derived NO acts on smooth muscle cells to induce vasodilation by increasing production of the second messenger cyclic guanosine monophosphate (cGMP) via activation of soluble guanylate cyclase (sGC) [11,12].

Recent years have seen substantial progress in the treatment of PAH, with the development of palliative therapies that target the NO, endothelin and prostacyclin signalling pathways to promote vasodilation. However, although these developments have improved outcomes for patients with PAH, survival rates and quality of life remain relatively low [13].

Treatment of PH with NO releasing agents such as nitrates failed to produce beneficial long-term effects, as in most cases negligible pulmonary vasodilatation was

counterbalanced by significant peripheral reduction in vascular resistance and reflex tachycardia [14], which are poorly tolerated by patients with severe PH. Inhaled NO is widely used as a short-term vasodilator to identify so-called responders to calcium channel blockers [15]. The long-term use of NO, however, is hampered by technical problems of administration, and life-threatening rebound PH can occur following interruption or discontinuation of NO inhalation [14]. An alternative therapeutic strategy targets downstream components of the NO signalling pathway by inhibiting phosphodiesterase-5, which regulates the conversion of the second messenger cGMP to GMP [16]. Sildenafil has been the lead substance in this group of agents, showing both acute and long-term beneficial effects in patients with PAH [17,18]. However, phosphodiesterase-5 inhibition is not effective in all patients with PH [19]. The full therapeutic potential of the NO signalling pathway therefore remains to be exploited.

Riociguat (BAY 63-2521) offers a new mode of action for the treatment of PAH: in preclinical studies, it has been shown to stimulate sGC directly, increasing the enzyme's activity independently of NO while also increasing sensitivity to low levels of NO. Treatment of two rodent models of pulmonary hypertension (PH) with riociguat reduced pulmonary arterial pressure and partially reversed cardiac hypertrophy and vascular remodelling [20]. The aim of this clinical study was to evaluate the short term safety profile, tolerability and efficacy of riociguat in patients with moderate to severe pulmonary hypertension.

Methods

Patients

Male and female patients aged 18–80 years were eligible for inclusion if they had a mean pulmonary vascular resistance (PVR) > 300 dyn.s/cm^5 and a diagnosis of PAH, distal chronic thromboembolic PH or PH associated with mild to moderate interstitial lung disease. Catheters for haemodynamic measurements were placed on clinical grounds independently of the trial. A 12 h washout period was observed for acute vasodilatory substances such as calcium channel blockers and phosphodiesterase-5 inhibitors.

Patients were excluded from the study if they had any of the following: pre-existing lung diseases (other than interstitial lung disease as defined in the protocol), significant left heart dysfunction, significantly impaired gas exchange (partial pressure of carbon dioxide in arterial blood [$PaCO_2$] > 55 mmHg), deficiencies of blood coagulation or evidence of latent bleeding risk, sickle cell anaemia, peripheral organ dysfunction or immunodeficiencies. Women with childbearing potential were excluded if they were not using a reliable contraceptive measure. Patients were also excluded if they had participated in another study during the thirty days preceding the current study, or if they had undergone previous therapeutic radiation of lung or mediastinum. During the trial, use of medication other than the investigational product was permitted only after consultation with the investigator, and all concomitant medications were documented.

Study design

Riociguat was administered in the morning after a fasting period of at least 8 h. After a first baseline evaluation lasting 30 min, every patient underwent a NO inhalation period of 10 min (10–20 ppm NO required for maximum vasodilatation), followed by

a second baseline period of 50 min. Haemodynamic and gas exchange variables were measured twice during the NO inhalation period and the second baseline period. BAY 63-2521 was administered orally in solution (unit of dosage: 0.5 mg/mL) after haemodynamic variables returned to baseline values.

In part A of the study, designed to identify the dose of riociguat which has maximal clinical effect without compromising safety and tolerability, four patients were given hourly incremental doses (0.5 + 1 + 1 mg = 2.5 mg [n = 2]; 1 + 2 + 2 mg = 5 mg [n = 2]). Dose titration was terminated if the mean arterial pressure fell below 60 mmHg or if the heart rate exceeded 140 bpm (Figure 1a). In part B, 1 mg and 2.5 mg single doses of riociguat were evaluated in five and ten patients respectively (Figure 1b). Measurements obtained for riociguat treatment were compared with peak intervention values for inhaled NO and post-NO intervention baseline values.

The study protocol and any substantial amendments were approved by the *Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)* and the Ethics Committee of the University of Giessen Medical Faculty. Each patient gave written informed consent.

Safety and tolerability

Safety and tolerability were evaluated using standard vital sign and laboratory biochemistry. The subjective tolerability of riociguat was evaluated by questioning the subjects about adverse events or by spontaneous reporting of adverse events. Clinical adverse events were classified according to their degree of severity (mild, moderate or severe), and it was also noted whether or not they were serious. Patients were assessed for 48 h following administration of riociguat.

Pharmacodynamics

The pharmacodynamic effects of the drug were assessed using Swan-Ganz catheterization, blood gas measurements and multiple inert gas elimination technique (MIGET) as described previously [21].

Swan-Ganz haemodynamic measurements included direct measurements such as mean right atrial pressure (RAm, mmHg), pulmonary arterial pressure (PAPsyst, PAPdiast, mPAP, mmHg), pulmonary capillary wedge pressure (PCWP, mmHg), heart rate (HR, BPM), systemic blood pressure (systolic, SBP; diastolic, DBP; arterial mean, SAP mean; mmHg), cardiac output (CO, L/min [average of 3 measurements, performed and calculated by CO thermo-dilution device]), body weight (kg) and height (cm). Calculated measurements included body surface area (BSA, m^2) = (weight [kg]$^{0.425}$) × (height [cm]$^{0.725}$) × 0.007184 (Dubois formula), PVR = 80 × (PAP - PCWP) / CO (dyn.sec/cm^5), systemic vascular resistance (SVR) = 80 × (SAP mean - RAm) / CO (dyn.sec/cm^5) and cardiac index = CO / BSA (L/min/m^2).

Blood gas analysis measurements included PaO_2 (mmHg), $PaCO_2$ (mmHg), arterial oxygen saturation (SaO_2 [%]), mixed venous oxygen tension (PvO_2 [mmHg]) and venous oxygen saturation (SvO_2 [%]).

MIGET measured total ventilation (V, L/min), total perfusion (Q, L/min), deadspace ventilation (percent of total ventilation), low V/Q perfusion (V/Q 0.001-0.1 in percent of total perfusion), normal V/Q perfusion (V/Q 0.1-10 in percent of total perfusion), ventilation-perfusion distribution (standard deviation of perfusion and standard deviation of ventilation) and intrapulmonary shunt flow (percent of total perfusion).

To assess the relationship between riociguat plasma concentration and the effect on PAP, SBP, PVR, SVR, and cardiac index (measured as the ratio to baseline), Spearman's rank correlation coefficients along with 95% confidence intervals were calculated for each parameter and all subjects.

Pharmacokinetics

Riociguat plasma concentrations were determined by HPLC-MS assay, using blood samples obtained at regular intervals up to 48 h after riociguat administration. Primary parameters included the following: area under the plasma concentration versus time curve from zero to infinity (AUC); AUC divided by dose (mg), expressed per kg body weight (AUC_{norm}); maximum drug concentration in plasma after single dose administration (C_{max}); and C_{max} divided by dose (mg), expressed per kg body weight ($C_{max,norm}$). Secondary parameters included time to reach maximum drug concentration in plasma (t_{max}), and half-life associated with terminal elimination ($t_{1/2}$). Apparent volume of distribution during terminal phase after oral administration (Vz/f) and total body clearance of drug from plasma after oral administration (CL/f) were also calculated.

The influence of riociguat dose on pharmacokinetic parameters was assessed by performing an explorative analysis of variance (including factor “dose”) on the log-transformed values of AUC_{norm} and $C_{max,norm}$ in the single-dose study.

Results

Patient demographics

Patient demographics are summarized in Supplementary Table 1 (available online). There was no clinically relevant difference in patient demographics between study part A and part B. Patients enrolled in part B showed no statistically significant differences in age, height, weight or body mass index between dose groups. The 1 mg dose group had a higher proportion of women than the 2.5 mg dose group. Medications used by patients prior to the study are presented in Supplementary Table 2.

Safety and tolerability

No serious adverse events occurred in this study (Table 1). Overall, six mild adverse events were documented in 4 of 19 patients, all of which had resolved by study completion. Three adverse events were considered to be drug-related and attributable to the pharmacological properties of the test compound.

Riociguat at a dose of ≤ 2.5 mg had no clinically relevant effects on vital signs, electrocardiograms or laboratory values, and no major changes were noted in blood gases (PaO_2, $PaCO_2$, blood oxygen saturation) or ventilation-perfusion matching. Riociguat was well tolerated up to 2.5 mg, whereas a total dose of 5 mg in part A caused asymptomatic hypotension in one patient. Therefore, a 2.5 mg dose was used in part B to demonstrate efficacy; 1 mg was chosen to test for the first effect level.

Pharmacodynamics

Baseline pharmacodynamic parameters are shown in Table 2. Inhaled NO led to small, non-significant reductions from baseline in mPAP, SBP, PVR, and SVR, and no relevant changes were observed for cardiac index or heart rate (Figure 2). mPAP,

SBP, PVR and SVR showed a similar maximum decrease in response to NO inhalation in both 1 mg and 2.5 mg dose groups.

Both 1 mg and 2.5 mg doses of riociguat caused clinically relevant and statistically significant reductions from baseline in mPAP, PVR, SBP and SVR to a similar extent. A clinically relevant and statistically significant increase in cardiac index was also observed with both doses (*P* between 0.0151 and <0.0001, Figure 2), whereas a significant increase in heart rate was only observed in the 2.5 mg dose group. Both doses of riociguat were superior to NO in reducing PVR, SBP and SVR and increasing cardiac index (*P* between 0.0220 and <0.0001, Figure 2), and the 2.5 mg dose was superior to NO in reducing mPAP (*P* = 0.0341; Figure 2a). Riociguat had a similar effect in patient subgroups with PAH or chronic thromboembolic PH, significantly increasing cardiac index to a greater extent than NO (Figure 3). Riociguat plasma concentrations correlated significantly with the reductions in mPAP, SBP, PVR and SVR, and the concomitant increase in cardiac index (Table 3, Figure 4a). Neither dose of riociguat produced any deterioration in gas exchange or ventilation/perfusion matching (as measured by MIGET), despite causing strong pulmonary vasodilation (Supplementary Table 3).

Pharmacokinetics

Following single-dose administration of riociguat solution, plasma concentrations of riociguat showed dose-dependent increases with pronounced inter-individual variability (Figure 4b). Peak concentrations of riociguat were reached after 0.25 to 1.5 h, and its half-life was between 10 and 12 h (Table 4). C_{max} and AUC values for riociguat suggested dose proportionality for the 1 mg and 2.5 mg doses (Table 4); this

was supported by analysis of variance results which showed that factor dose had no influence on either parameter (AUC_{norm}, $P = 0.7559$; $C_{max,norm}$, $P = 0.6128$).

Discussion

In this proof-of-concept study in patients with PAH, distal chronic thromboembolic PH or PH associated with mild to moderate interstitial lung disease (mean PVR > 300 dyn.s/cm^5), doses of 1 mg or 2.5 mg of riociguat significantly reduced PVR and also improved PAP and CI in a concentration-dependent manner. Both doses demonstrated greater potency and duration of action than inhaled NO, which had a small, statistically insignificant effect in our study, consistent with the fact that a substantial proportion of PH patients does not respond to NO inhalation [22]. Neither dose of riociguat produced any deterioration in gas exchange or ventilation/perfusion matching, despite strong pulmonary vasodilation.

Riociguat had a favourable safety profile, with a single dose of 2.5 mg being well tolerated. A total dose of 5 mg administered in hourly increments gave rise to asymptomatic hypotension in one patient in part A; therefore, subsequent tests were limited to a maximum dose of 2.5 mg. The favourable safety profile observed in the present study is in agreement with a previous phase I study, in which riociguat at oral doses of up to 5.0 mg was well tolerated by healthy male volunteers [23].

Oral riociguat was effective in the current study, thus offering the patient a simple and convenient mode of administration. Oral therapies currently approved for the treatment of PAH comprise ERAs and phosphodiesterase-5 inhibitors. Although these therapies have helped to improve the prognosis for patients with PAH, survival rates remain relatively poor [13], suggesting that a new approach is required. In addition,

ERAs are associated with an increased risk of hepatotoxicity [24]. Riociguat did not demonstrate any hepatotoxicity in the current short-term study, supporting previous work in experimental models and human volunteer studies [23]: no significant abnormalities in laboratory values were recorded in association with the trial, apart from one case of slightly elevated glutamate dehydrogenase, pre-existent in a patient who had been treated with ERAs before the study and who had a history of intermittent increases of liver enzymes.

The phosphodiesterase-5 inhibitor sildenafil is widely-used and well-tolerated as a therapy for PAH, and has demonstrated a degree of pulmonary selectivity despite being administered orally [25], presumably due to the relatively high expression of phosphodiesterase-5 in the lung [26]. However, its efficacy is dependent on the presence of an intact NO-sGC-cGMP axis [27], and may be limited in the presence of low levels of NO; sildenafil blocks degradation of cGMP and thus depends on the presence of NO stimulating sGC, in contrast to riociguat which can increase cGMP synthesis in the absence of NO. Although various doses of sildenafil (20–80 mg TID) were used in the SUPER-1 trial [18], and the bulk of long-term experience with this drug is with doses of more than 20 mg TID, agencies have only approved the lowest dose of 20 mg TID for long-term treatment, which could be too low for some patients [16]. The synergistic action of riociguat with low levels of NO may provide a means to increase cGMP levels and thus promote vasodilation in combination with sildenafil or in patients who do not respond to sildenafil, while also ensuring maintenance of ventilation/perfusion matching.

Riociguat significantly reduced SBP and SVR in the current study and therefore did not demonstrate pulmonary selectivity although, interestingly, the systemic vasodilation was not accompanied by any relevant side effects (Table 1). This may

have been due to a compensatory increase in cardiac output. Although the observed systemic effects of riociguat were asymptomatic in this short-term study of supine patients, its long-term effect on mobile patients may be more significant and must be examined in future studies.

The current study has certain limitations, for example the small and heterogeneous patient population exposed to the drug, lack of a placebo group and the observation of short-term effects only. In addition, plasma cGMP levels, which would have provided information regarding the direct effect of riociguat on sGC activity, were not assessed. Nevertheless, the results of the study present riociguat as a promising novel therapeutic principle which warrants further investigation.

Direct NO-independent stimulation of sGC and sensitization of sGC to low levels of endogenous NO may offer distinct advantages over current therapeutic approaches and opens access to a completely new class of drugs for cardiovascular indications [28]. Riociguat stimulates the NO target sGC directly to create a strong vasodilatory effect irrespective of integrity of endothelial function and NO production, and also acts synergistically in the presence of NO, thus offering a new mode of action for the treatment of PH. In this study, convenient oral doses of riociguat demonstrated efficacy, tolerability and durable action, with no deterioration in gas exchange. Comparison of this promising drug with established therapies such as sildenafil, and assessment of its efficacy as an add-on therapy or in patients who do not respond to existing treatments, would be of great interest in future studies.

In conclusion, this study demonstrates the efficacy of riociguat in lowering pulmonary vascular resistance and improving cardiac function in patients with moderate-to-severe PH. As a representative of a new class of drugs hitherto not evaluated in cardiovascular disease, riociguat therefore offers great therapeutic potential as a

treatment for patients with pulmonary vascular disorders. The encouraging results of the current acute haemodynamic study warrant further long-term controlled clinical trials in this field. A multi-centre phase II trial has recently been initiated to address this issue.

Acknowledgements

Parts of the doctoral thesis of Desire Bolköw are incorporated in this report. Dr Claire Mulligan from Oxford PharmaGenesis™ provided editorial assistance funded by Bayer HealthCare AG.

References

1. Gaine SP, Rubin LJ. Primary pulmonary hypertension. *Lancet* 1998; 352: 719-25.
2. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 1991; 115: 343-9.
3. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 2006; 173: 1023-30.
4. Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. *Eur Respir J* 2007; 30: 104-9.
5. Speich R, Jenni R, Opravil M, Pfab M, Russi EW. Primary pulmonary hypertension in HIV infection. *Chest* 1991; 100: 1268-71.
6. Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J, Kahan A, Cabane J, Frances C, Launay D, Mouthon L, Allanore Y, Tiev KP, Clerson P, de Groote P, Humbert M. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum* 2005; 52: 3792-800.
7. Plastiras SC, Karadimitrakis SP, Kampolis C, Moutsopoulos HM, Tzelepis GE. Determinants of pulmonary arterial hypertension in scleroderma. *Semin Arthritis Rheum* 2007; 36: 392-6.
8. Billy-Brissac R, Blanchet-Deverly A, Etienne-Julan M, Foucan L. Pulmonary hypertension in an adult sickle cell population in Guadeloupe. *Int J Cardiol* 2008.
9. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K, Brown B, Coles WA, Nichols JS, Ernst I, Hunter LA, Blackwelder WC, Schechter AN, Rodgers GP, Castro O, Ognibene FP. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med* 2004; 350: 886-95.

10. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. *Circulation* 2006; 114: 1417-31.
11. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* 1987; 84: 9265-9.
12. Arnold WP, Mittal CK, Katsuki S, Murad F. Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations. *Proc Natl Acad Sci U S A* 1977; 74: 3203-7.
13. Naeije R, Huez S. Expert opinion on available options treating pulmonary arterial hypertension. *Expert Opin Pharmacother* 2007; 8: 2247-65.
14. Atz AM, Adatia I, Wessel DL. Rebound pulmonary hypertension after inhalation of nitric oxide. *Ann Thorac Surg* 1996; 62: 1759-64.
15. Sitbon O, Brenot F, Denjean A, Bergeron A, Parent F, Azarian R, Herve P, Raffestin B, Simonneau G. Inhaled nitric oxide as a screening vasodilator agent in primary pulmonary hypertension. A dose-response study and comparison with prostacyclin. *Am J Respir Crit Care Med* 1995; 151: 384-9.
16. Ghofrani HA, Osterloh IH, Grimminger F. Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat Rev Drug Discov* 2006; 5: 689-702.
17. Ghofrani HA, Wiedemann R, Rose F, Olschewski H, Schermuly RT, Weissmann N, Seeger W, Grimminger F. Combination therapy with oral sildenafil and inhaled iloprost for severe pulmonary hypertension. *Ann Intern Med* 2002; 136: 515-22.
18. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med* 2005; 353: 2148-57.
19. Bhatia S, Frantz RP, Severson CJ, Durst LA, McGoon MD. Immediate and long-term hemodynamic and clinical effects of sildenafil in patients with pulmonary arterial hypertension receiving vasodilator therapy. *Mayo Clin Proc* 2003; 78: 1207-13.
20. Schermuly R, Stasch JP, Pullamsetti SS, Middendorff R, Mueller D, Schlüter KD, Dingendorf A, Hackemack S, Kolosionek E, Kaulen C, Dumitrascu R, Weissmann N, Mittendorf J, Klepetko W, Seeger W, Ghofrani HA, Grimminger F. Expression and function of soluble guanylate cyclase in pulmonary arterial hypertension. *Eur Respir J* 2008; 32: 881-91.
21. Ghofrani HA, Wiedemann R, Rose F, Schermuly RT, Olschewski H, Weissmann N, Gunther A, Walmrath D, Seeger W, Grimminger F. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. *Lancet* 2002; 360: 895-900.
22. Klinger JR, Thaker S, Houtchens J, Preston IR, Hill NS, Farber HW. Pulmonary hemodynamic responses to brain natriuretic peptide and sildenafil in patients with pulmonary arterial hypertension. *Chest* 2006; 129: 417-25.
23. Frey R, Mück W, Unger S, Artmeier-Brandt U, Weimann G, Wensing G. Single-dose pharmacokinetics, tolerability and safety of the soluble guanylate cyclase stimulator BAY 63-2521; an ascending-dose study in healthy male volunteers. *J Clin Pharmacol* 2008; 48: 926-934.
24. Humbert M, Segal ES, Kiely DG, Carlsen J, Schwierin B, Hoeper MM. Results of European post-marketing surveillance of bosentan in pulmonary hypertension. *Eur Respir J* 2007; 30: 338-44.

25. Ghofrani HA, Voswinckel R, Reichenberger F, Olschewski H, Haredza P, Karadas B, Schermuly RT, Weissmann N, Seeger W, Grimminger F. Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension: a randomized prospective study. *J Am Coll Cardiol* 2004; 44: 1488-96.
26. Corbin JD, Beasley A, Blount MA, Francis SH. High lung PDE5: a strong basis for treating pulmonary hypertension with PDE5 inhibitors. *Biochem Biophys Res Commun* 2005; 334: 930-8.
27. Michelakis ED. The role of the NO axis and its therapeutic implications in pulmonary arterial hypertension. *Heart Fail Rev* 2003; 8: 5-21.
28. Evgenov OV, Pacher P, Schmidt PM, Hasko G, Schmidt HH, Stasch JP. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat Rev Drug Discov* 2006; 5: 755-68.

Figure legends

Figure 1. Treatment schedules for evaluation of riociguat in patients with pulmonary hypertension. (A) Identification of maximum tolerated dose. (B) Evaluation of 1 mg and 2.5 mg doses. NO, inhaled nitric oxide.

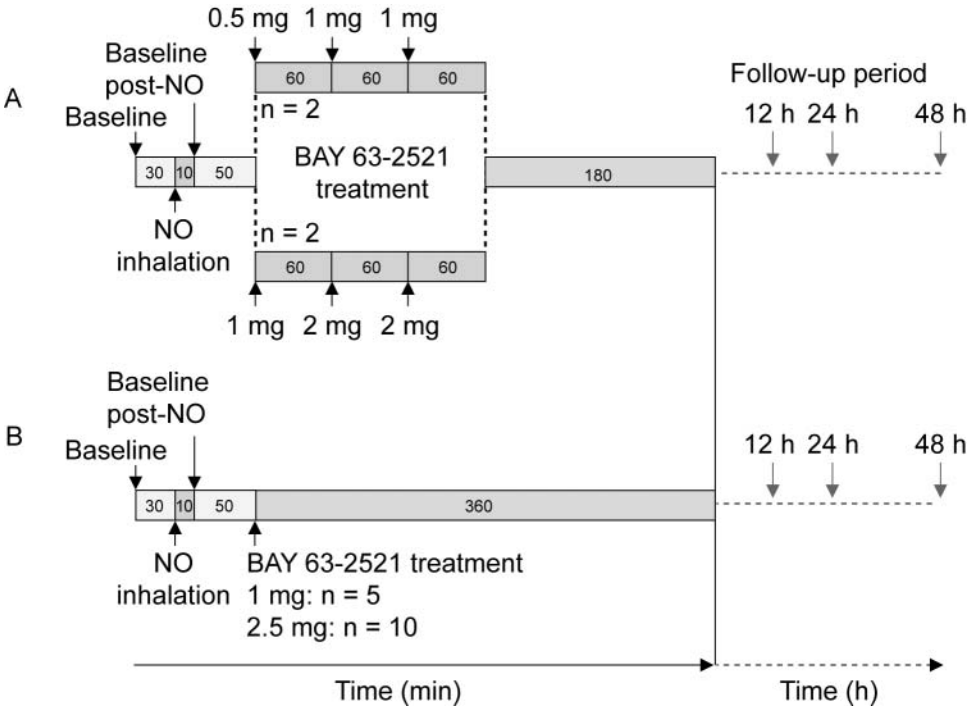


Figure 2. Changes in haemodynamic parameters following a single dose of riociguat, compared with inhaled nitric oxide. (A–D) Percentage decrease from baseline of mean pulmonary arterial pressure (A), systolic blood pressure (B), pulmonary vascular resistance (C), and systemic vascular resistance (D). (E–F) Percentage increase from baseline of cardiac index (E) and heart rate (F). Horizontal lines indicate point estimates (least-squares means) in each case. Statistical significance was measured by the F statistic (*$P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$; § $P < 0.0001$). Rio, riociguat; NO, inhaled nitric oxide.

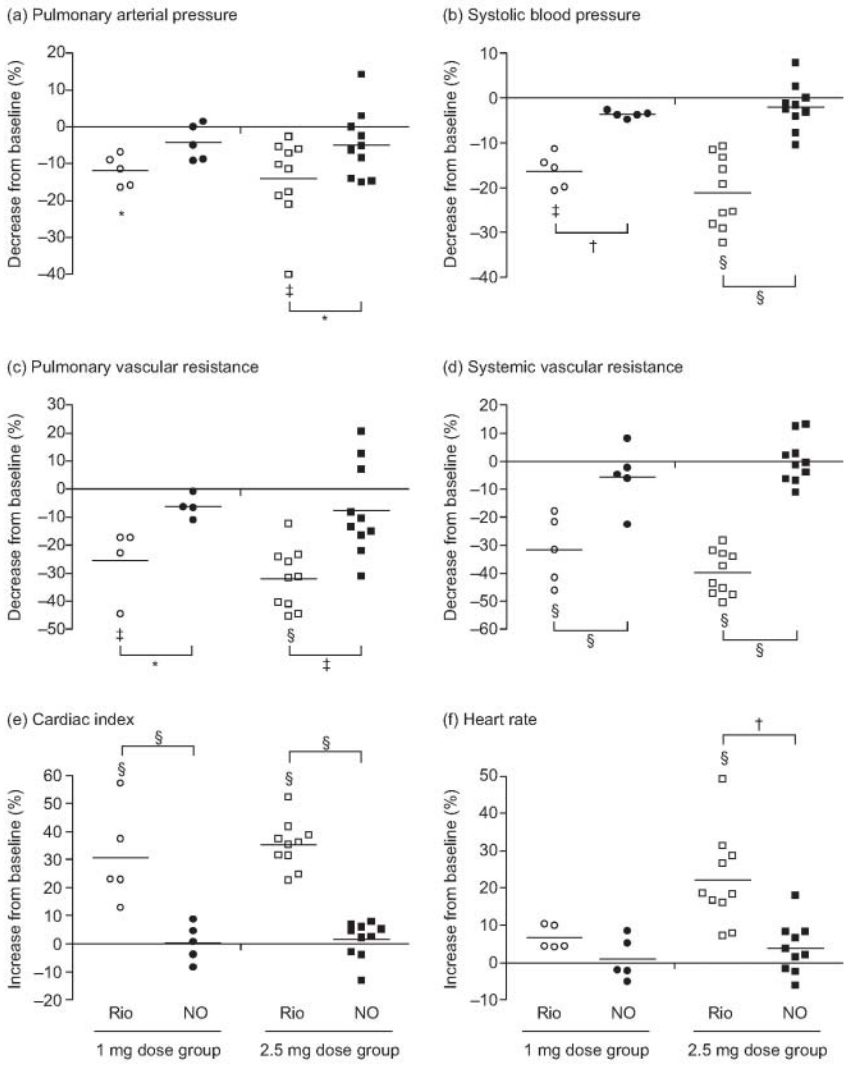


Figure 3. Change from baseline of cardiac index in patients with distal chronic thromboembolic pulmonary hypertension (CTEPH) or pulmonary arterial hypertension (PAH) following a single dose of riociguat, compared with inhaled nitric oxide. Horizontal lines indicate point estimates (least-squares means) in each case.

Statistical significance was measured by the F statistic (*$P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$; § $P < 0.0001$). No significant differences in response to drug were observed between the two disease entities. Rio, riociguat; NO, inhaled nitric oxide.

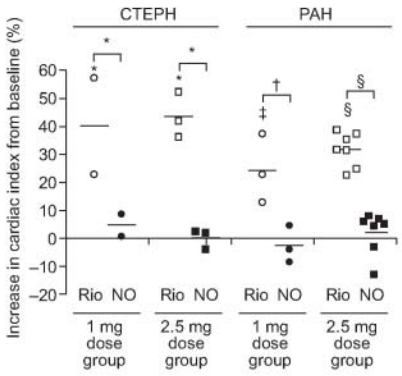
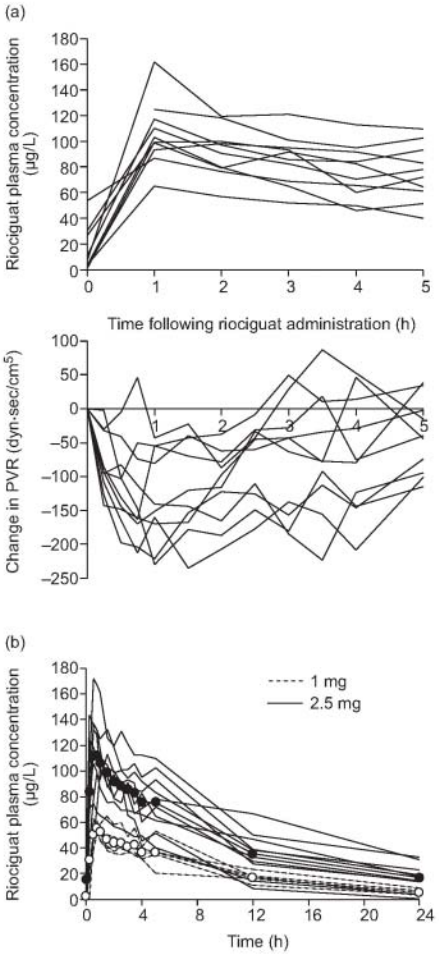


Figure 4. Pharmacokinetic analysis of riociguat following a single oral dose. (A) Comparison of riociguat plasma concentration with changes in pulmonary vascular resistance (PVR) from baseline over time. (B) Study Part B – riociguat plasma concentrations following a single oral dose of 1 mg and 2.5 mg riociguat solution. Data are shown for each individual patient, and filled and empty circles indicate geometric means for the 2.5 mg and 1 mg dose groups, respectively. Subjects valid for pharmacokinetic analysis; n = 15.


(a)
Riociguat plasma concentration (μg/L)
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
Time following riociguat administration (h)
Change in PVR (dyn-sec/cm5)
100
50
0
−50
−100
−150
−200
−250
1
2
3
4
5
(b)
Riociguat plasma concentration (μg/L)
1 mg
2.5 mg
0
4
8
12
16
20
24
Time (h)

Tables

Table 1. Treatment-emergent adverse events and possible relationship to study drug.

	iNO	**Riociguat**			
		Incremental dose		**Single dose**	
		0.5 + 1 + 1 mg	**1 + 2 + 2 mg**	**1 mg**	**2.5 mg**
n	19	2	2	5	10
Adverse events* (%)	0 (0)	1 (50)	1 (50)	1 (20)	1 (10)
Renal and urinary disorders:					
	–	Micturition urgency†	Urinary retention†	–	–
Vascular disorders:					
	–	Hot flush‡	–	–	–
Nervous system disorders:					
	–	–	–	Dizziness‡	–
Respiratory, thoracic and mediastinal disorders:					
	–	–	–	Cough†	Nasal congestion‡

iNO, inhaled nitric oxide; *all adverse events were mild in severity; †not drug related; ‡at least possibly drug related.

Table 2. Baseline pharmacodynamic characteristics in study part B.

Parameter	**Riociguat**	
	1 mg group (n = 5)	**2.5 mg group (n = 10)**
mPAP (mmHg)	55.4 ± 16.6	42.1 ± 11.3
PVR (dyn·s/cm^5)	1028 ± 491	566 ± 209
SBP (mmHg)	147 ± 25	133 ± 20
SVR (dyn·s/cm^5)	2127 ± 407	1324 ± 335
Cardiac index (L/min/m^2)	2.17 ± 0.18	2.74 ± 0.82
Heart rate (bpm)	84 ± 12	73 ± 10

bpm, beats per minute; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; SBP, systolic blood pressure; SVR, systemic vascular resistance.

Table 3. Statistics on Spearman correlation between riociguat drug concentration and PAP, SBP, PVR, SVR, and cardiac index (subjects valid for pharmacokinetic and pharmacodynamic analysis, n=19).

Parameter	Unit	Correlation	95% confidence interval	Two-sided *P*>|Z|
Pulmonary arterial pressure (PAP)	mmHg	-0.2550	[-0.3827 ; -0.1274]	<0.0001
Systolic blood pressure (SBP)	mmHg	-0.5569	[-0.6531 ; -0.4607]	<0.0001
Pulmonary vascular resistance (PVR)	dyn.s/cm^5	-0.4733	[-0.5815 ; -0.3650]	<0.0001
Systemic vascular resistance (SVR)	dyn.s/cm^5	-0.5910	[-0.6879 ; - 0.4942]	<0.0001
Cardiac index	L/min/m^2	0.4543	[0.3411 ; 0.5674]	<0.0001

Table 4. Study Part B – pharmacokinetic parameters of riociguat in plasma following single-dose administration of 2.5 mg and 1 mg riociguat (subjects valid for pharmacokinetic and pharmacodynamic analysis, n = 15).

Parameter	**Unit**	**Riociguat 1 mg (n=5)**			**Riociguat 2.5 mg (n=10)**		
		Geometric mean	**CV (%)**	**Range**	**Geometric mean**	**CV (%)**	**Range**
AUC	µg.h/L	602.3	14.9	456.5 - 749.6	1411	39.2	597.5 - 3121
C_{max}	µg/L	59.43	5.9	53.49 - 65.05	119.4	16.1	74.69 - 172.4
t_{max}	h	0.750*		0.500 - 1.500	0.500*		0.250 - 1.500
$t_{1/2}$	h	9.953	8.6	8.737 - 12.14	11.65	38.6	4.680 - 28.58
Vz/f	L/kg	0.354	7.3	0.307 - 0.393	0.378	20.7	0.005 - 0.609
CL/f	L/h	1.660	14.9	1.334 - 2.191	1.771	39.2	0.801 - 4.184

*Median; AUC, area under plasma concentration / time curve from zero to infinity after single dose; CL/f, total body clearance of drug from plasma calculated after oral administration; C_{max}, maximum drug concentration in plasma after single-dose administration; CV, coefficient of variance (geometric); $t_{1/2}$, terminal elimination half-life; t_{max}, time to reach maximum drug concentration in plasma; Vz/f, apparent volume of distribution during terminal phase after oral administration.