Tyrosine Kinase Inhibitor BIBF1000 does not hamper right ventricular pressure adaptation in rats

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ABSTRACT:

BIBF1000 is a small molecule tyrosine kinase inhibitor targeting Vascular Endothelial Growth Factor Receptor (VEGFR), Fibroblast Growth Factor Receptor (FGFR) and Platelet Derived Growth Factor Receptor (PDGFR) and is a powerful inhibitor of fibrogenesis. BIBF1000 is very similar to BIBF1120 (Nintedanib), a drug recently approved for the treatment of Idiopathic Pulmonary Fibrosis (IPF). A safety concern pertaining to VEGFR, FGFR and PDGFR inhibition is the possible interference with right ventricular (RV) responses to an increased afterload, which could adversely affect clinical outcome in IPF patients who developed pulmonary hypertension. We tested the effect of BIBF1000 on the adaptation of the RV in rats subjected to mechanical pressure overload.

BIBF1000 was administered for 35 days in pulmonary artery banded (PAB) rats. RV adaptation was assessed by echocardiography, pressure volume loop analysis, histology and determination of atrial natriuretic peptide (ANP) expression.

BIBF1000 treatment resulted in growth attenuation but had no effects on RV function after PAB, given absence of changes in cardiac index, end systolic elastance, connective tissue disposition and capillary density. We conclude that in this experimental model of increased afterload, combined VEGFR, FGFR and PDGFR inhibition does not hamper RV adaptation to pressure overload.

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Key words: experimental Pulmonary Hypertension, pulmonary artery banding, tyrosine kinase inhibitor, cardiac remodeling, BIBF1000.
**New and noteworthy:**

Based on this translational rat study, it is suggested that cardiac function will not worsen when Nintedanib is used for treatment of Idiopathic Pulmonary Fibrosis, even after the development of associated Pulmonary Hypertension.
Introduction

Idiopathic Pulmonary Fibrosis (IPF) is characterized by progressive fibroblast/myofibroblast accumulation resulting in increased deposition of extracellular matrix components like collagen in the lungs. These pathogenic mechanisms are partly driven by growth factor signaling through Vascular Endothelial Growth Factor (VEGF), Platelet Derived Growth Factor (PDGF) and Fibroblast Growth Factor (FGF) receptors (2, 17, 40). BIBF1000, a potent tyrosine kinase inhibitor of VEGF, PDGF and FGF receptor signaling (7), attenuated pulmonary fibrosis in an animal model of lung fibrosis resembling aspects of IPF (13). Nintedanib (BIBF1120) and BIBF1000 are indolinone derivatives designed during the same chemical lead optimization program and are very similar with respect to the kinase specificity profile (7, 13). Nintedanib has anti-tumor activity and like BIBF1000 anti-fibrotic activity in animal models of IPF (16, 21, 38, 45). Because Nintedanib slows the decline in lung function in patients with IPF (36), the drug was recently approved for the treatment of IPF in the United States and Europe (46).

IPF is complicated by pulmonary hypertension (PH) in about 45% of patients (12, 39). To adapt to the increased pulmonary vascular pressures, the right ventricle (RV) undergoes progressive adaptation through increased contractility and hypertrophy, processes involving VEGF, PDGF and FGF signaling. Upregulation of VEGF in the pressure overloaded heart is required to maintain capillary density and systolic function (25, 37). Intact PDGF signaling contributes to cardiomyocyte survival and cardiac adaptation through activation of cardiomyocyte progenitor cells (6, 47). In models of acute myocardial infarction, enhanced PDGF signaling is cardioprotective (14, 22). FGF induces hypertrophic responses in cardiomyocytes, for example by
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activation of mitogen-activated protein kinase (MAPK) (24, 32). FGF-2 deficient mice develop dilated cardiomyopathy and do not show compensatory hypertrophy when hypertensive (32). Because cardiac remodeling involves VEGF, PDGF and FGF receptor signaling, the question may be raised whether BIBF1000 and nintedanib could hamper cardiac adaptation. This question is critically important because RV function is an important determinant of prognosis in IPF (18, 41, 44).

The aim of this study was to determine whether VEGF, PDGF and FGF receptor inhibition by BIBF1000 affects compensatory remodeling of the RV in response to relatively mild pressure overload. When rats are exposed to a moderate degree of mechanical pressure overload through pulmonary artery banding (PAB), the right ventricle normally adapts without developing RV failure. The PAB model has been used previously to detect potential cardiotoxicity of new PAH drugs (9).
Material and methods

Animal model

Thirty-two male Sprague Dawley rats (Crl:CD(SD), Charles River, Sulzfeld, Germany), 180-200 grams body weight (BW) underwent PAB (n=16), or sham surgery (n=16) as described previously (10). Via a left thoracotomy, a silk suture was tied tightly around an 18-gauge needle alongside the pulmonary artery. After subsequent rapid removal of the needle, a fixed constricted opening was created in the lumen equal to the cross-sectional area of the needle. One week after surgery, animals were randomized to vehicle or BIBF1000 treatment (n=8/group). Clinical signs and body weights were measured daily. The study was approved by the local Animal Welfare committee (VU-Fys 11-11).

Test substance formulation and dosing

The citrate salt of BIBF1000 (IUPAC-code 35113576) (Boehringer Ingelheim Pharma, Biberach Germany) was dissolved in deionized water (MilliQ, Millipore) to reach a concentration of 10 mg/mL (on basis of the free base) and stored at room temperature to a maximum of 7 days. Based on the toxicokinetic profile (data in courtesy of Boehringer Ingelheim), BIBF1000 was administered at a dose of 50 mg/kg body weight once daily by oral gavage for 35 days. Dose calculation was adjusted to the individual body weights twice weekly. As the dosing period started 7 days after surgery, there was a minor RV systolic pressure difference present at start between the PAB group and the sham group.
Effect of BIBF1000 on PDGF-R and FGF-R signaling in heart tissue

BIBF1000 was administered to 180-200 g male Sprague Dawley rats (Charles River, Kissleg, Germany) by gavage at 50 mg/kg (n = 3 per group). 120 or 240 minutes after dosing, the animals were anesthetized with 60 mg/kg i.p. pentobarbital sodium and 3 mg/kg i.m. ketamin. Recombinant rat (Rr) PDGF-BB (50 µg/animal, ProSpec-Tany; TechnoGene Ltd., Ness-Ziona, Israel) or Rr FGF2 (100 µg/animal, Prospec-Tany; TechnoGene Ltd., Ness-Ziona, Israel) or vehicle was administered intravenously, and 5 and 10 minutes after PDGF-BB or bFGF stimulation, hearts were excised and snap frozen in liquid nitrogen.

Echocardiography

On the day of necropsy, animals received isoflurane 2-3% in 1:1 O₂/air mixture (60% oxygen) for anesthesia and underwent echocardiography using a ProSound system (Prosound SSD-4000) equipped with a 13-Mhz linear transducer (UST-5542, Aloka, Tokyo, Japan), as described previously (30). The following parameters were acquired: cardiac index (CI), tricuspid annular plane systolic excursion (TAPSE), RV wall thickness (RVWT) and RV end diastolic diameter (RVEDD).

Hemodynamics and pressure volume (PV)-loops

Subsequently, after echocardiography blood was sampled and the hematocrit was determined by centrifugation and fractional measurement. The chest was opened through the diaphragm. RV catheterization was performed using a Millar catheter (AD instruments, Bella Vista, Australia) for measuring right ventricular systolic pressure (RVSP), left ventricular systolic pressure (LVSP) and pressure-volume.
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measurements. For collecting end systolic elastance (EES), end diastolic elastance (EED) and arterial elastance (EA), the vena cava was occluded with a tightened string placed around the abdominal vena cava, as described previously (30).

Histological and morphometric analyses

After necropsy, cardiac tissues and organs were weighed and processed for histology. Organ weights were corrected for tibia length. Tissues were fixed in formalin and embedded in paraffin. Four micron slices of heart tissue were prepared and stained. To assess the presence of connective tissue, a Masson’s trichrome stain was performed. CD31 and CD45 immunohistochemical stains were performed to evaluate capillary density and inflammation, respectively (CD31: M-20, (Santa Cruz Biotechnology, Dallas, Texas) / CD45: H-230 (Santa Cruz Biotechnology, Dallas, Texas), 2nd antibody Alexa Fluor 488 anti-goat, (ThermoFisher Scientific, Waltham, MA, USA), with WGA Alexa fluor 555 conjugate (ThermoFisher Scientific, Waltham, MA, USA) for cardiomyocyte staining and Slowfade Gold Antifade Mountant with DAPI (ThermoFisher Scientific, Waltham, MA, USA) for nuclei staining).

Protein expression

According to the supplier’s manual (Santa Cruz Biotechnology, inc., Dallas, Texas), Western blots on RV homogenizations were performed for ANP (ANP(N-20):sc-18811, 1:200, p-ERK (p-ERK(E-4):sc-7383), ERK (ERK2(H-9):sc271451), p-Akt (p-Akt(Thr 308):sc-135650 and Akt (Akt(C-20):sc-1618) (all from Santa Cruz Biotechnology, inc., Dallas, Texas), with corresponding 2nd antibody, 1:5000 (DAKO, Glostrup, Denmark). Novex ECL chemiluminescent (Invitrogen, Carlsbad, California)
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was used for protein detection. To confirm the target engagement by BIBF1000 in heart tissue, Western blots were used probed with antibodies directed against GAPDH, phosphorylated PDGFRα and β (Tyr849/Tyr857), and phosphorylated extracellular signal-regulated kinases 1/2 (pERK1/2, Thr202/Tyr204), as downstream marker for FGFR inhibition (all Cell Signaling Technologies, Danvers, MA). Optical densities were measured and standardized with β-actin (A3854, 1:20000, Sigma, St. Louis, Missouri). The ANP blot was confirmed with an ELISA (ANP(NPPA) Rat ELISA kit (ab108797) Abcam plc., Cambridge, UK).

Statistical analyses

All data are presented as mean ± S.E.M. of n animals. Parametric variables were compared between groups using appropriate ANOVA with Bonferroni post-hoc tests (GraphPad Prism, GraphPad Software Inc, La Jolla, CA USA.). A p-value of less than 0.05 was considered significant.
RESULTS

Inhibition of the PDGF-R and FGF-R by BIBF1000

At 50 mg/kg BIBF1000 inhibited the PDGF- and FGF-stimulated phosphorylation of the PDGF receptor and the downstream molecule of FGF signaling, ERK1/2 in heart tissue, respectively (Figure 1AB). Inhibition of the VEGF-receptor was not measured because a validated method could not be established. However, inhibition of the VEGFR phosphorylation is expected to be similar because BIBF1000 exerts similar potency on the VEGFR compared to PDGFR and FGFRs.

Body weight gain and clinical signs

Increasing with time BIBF1000 treatment resulted in reduced body weight gain in sham treated (Figure 2A) and PAB-treated animals (Figure 2B). One BIBF1000-treated PAB-animal underwent necropsy one day before its scheduled necropsy due to significant weight loss and clinical signs (pilo erection and ruffled coat/ungroomed).

Echocardiography

PAB resulted in an increase of RVWT (Figure 3A) and RVEDD (Figure 3B) compared to sham treated animals. BIBF1000 did not significantly reduce the increased RVWT and RVEDD but led to a trend toward a reduction. No differences were found between the different treatment groups in TAPSE (Figure 3C) or CI (Figure 3D).

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RVSP, EES and EA (Figure 4A-C) were significantly increased in the PAB-treated animals. BIBF1000 did not significantly change the increase in RVSP, EES and EA. Ventriculo-arterial coupling (EES/EA), EED, stroke volume index (SVI) and LVSP (Figure 4D-G) were not affected by either PAB or BIBF1000 treatment.

Necropsy

The hematocrit remained within physiological ranges (Figure 5A). Heart weight was increased in the PAB-treated animals, due to an increase of both right ventricular weight (RV/tibia) (Figure 5B) and, to lesser extent, left ventricular plus septum weight ((LV+S)/tibia) (Figure 5C). Heart weights were normalized with the tibia length to account for growth retardation. The ratio in RV/(LV+S) increased dramatically in the PAB-treated animals describing severe right ventricular hypertrophy (Figure 5D). In sham-treated as well as PAB-treated animals BIBF1000 induced a trend towards a decrease in RV/(LV+S) ratio indicating a possible reduction in right heart hypertrophy.

Histological and morphometric analyses of the heart

PAB induced an increase in cardiomyocyte cross sectional area (CSA), indicating hypertrophy. BIBF1000 treatment significantly prevented the increase in CSA (Figure 6A). The number of capillaries per cardiomyocyte was increased after PAB in vehicle-treated rats, but not in BIBF1000-treated rats (Figure 6B). In comparison to the vehicle-treated animals with PAB the BIBF1000 treatment did not alter the number of capillaries per mm² (Figure 6C). No differences were seen among groups regarding amounts of connective tissue (Figure 6D) or inflammation (Figure 6E).
Protein expression

PAB resulted in a non-significant increase in ANP protein expression in vehicle treated rats, but not in BIBF1000-treated rats (Figure 7A-B). Basal activation and expression of ERK and Akt at the end of the experiment were not significantly different between BIBF1000-treated and vehicle treated PAB groups (Figure 7A-B).
DISCUSSION

Our study shows that VEGF, FGF and PDGF receptor inhibition by BIBF1000 does not adversely affect the morphology or RV function of rats subjected to PAB. TAPSE, ESP, EES and CI were not different between groups. The load independent index of RV function EES/EA was not significantly different between groups. BIBF1000 treatment of PAB rats resulted in a trend toward less RV dilatation and less RV hypertrophy. The number of capillaries per surface area was not altered. As a marker for cardiac stress, ANP trended to be elevated in vehicle-treated PAB-animals compared to sham animals, and this increase trended to be lower in the BIBF1000-treated PAB-animals. Because the ANP levels did not change in a statistically significant manner, it remains doubtful whether BIBF1000 may affect ANP expression. No alteration in activation and expression of ERK, Akt nor connective tissue disposition were observed after chronic BIBF1000 administration in PAB rats, which was probably due to the fact that the presence and degree of collagen is minimal in this experimental model of adaptive RV hypertrophy (10). Although, BIBF1000 is able to inhibit activation of ERK1/2 as a downstream target of the activated FGFR, there is no chronic effect on the basal activation of ERK 1/2 was observed in the PAB model. This could be due to the fact that the PAB model is not increasing the transcription of ERK (5). In summary, these findings suggest that it would be unlikely that inhibition of VEGFR, FGFR and PDGFR, either by BIBF1000 or by the very similar indolinone derivative nintedanib, results in a deterioration of RV function in IPF patients with secondary pulmonary hypertension. However, a clinical study to confirm these effects in human IPF with PH is warranted.
IPF is a progressive disease characterized by marked, patchy fibrosis in the lung parenchyma and loss of pulmonary function (33). In the pathophysiology of the disease, increased expression of FGF, PDGF and VEGF have been implicated (11, 23, 46). PH develops in approximately 45% of IPF patients and severely impacts functional status (12, 39). For example, the need for additional oxygen (L/min) was increased by 26% of the patients with PH in IPF and the cardiac index was decreased when mean pulmonary artery pressure exceeded 40 mmHg (39).

Importantly, RV function is the most conclusive determinant of prognosis in IPF patients with secondary PH (44). The existence of PH in patients with IPF increases the 1-year mortality to 28% compared to 5.5% in IPF patients without PH (28, 39). Currently no treatment targeting PH in patients with IPF is approved (12). Hence, it is of paramount importance that treatment options approved for patients with IPF have no detrimental effects on RV function. This safety concern could particularly apply to nintedanib, which was recently approved for the treatment of IPF.

At the start of our study a dosing rationale was available for BIBF1000 in rats (7) but not for the very similar BIBF1120 (nintedanib) which is now approved for the treatment of IPF in US and the EU (36). BIBF1000 at 50 mg/kg attenuated the deposition of collagen and the expression of pro-fibrotic genes in an animal model of bleomycin-induced lung fibrosis (7). Nintedanib and BIBF1000 are very similar with respect to their kinase specificity and potency (4, 5). The combined inhibitory activity of FGFR, PDGFR and VEGFR by both compounds is assumed to target lung fibrosis (5, 9, 11). FGFR1 and 2 are expressed on various cells in the lungs of patients with IPF (23), and FGF-2 stimulates proliferation of lung fibroblasts from patients with IPF (20). In vivo abrogation of FGF signaling reduces bleomycin-induced pulmonary fibrosis and improves survival in bleomycin-treated mice (48). PDGF was shown to
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have an important role in fibroblast/ myofibroblasts proliferation, migration and survival and acts as a stimulator of collagen synthesis (3, 11, 26, 31). Inhibition of PDGFR with specific compounds reduced pulmonary fibrosis in various animal models of lung fibrosis (1, 4, 29, 35, 43). VEGFR was assumed to be implicated in aberrant neovascularization (15, 34) and anti-VEGF gene therapy was shown to attenuate bleomycin-induced fibrosis in mice (19). In the heart, the same growth factors play a key role in cardiac adaptation and remodeling (16, 21, 24, 32).

Inhibition of VEGF and PDGF signaling could lead to vascular rarefaction (8, 14, 22, 25, 37), while PDGF and FGF inhibition could theoretically lead to an insufficient RV hypertrophic response (8, 24, 32). As such, the use of nintedanib in patients with IPF complicated by PH could be potentially detrimental to RV function. At the time of our study, we had no access to nintedanib because the drug was still being evaluated in the registration process for the treatment of IPF. As first pre-clinical evidence, our translational study shows no cardiac dysfunction in PAB rats after treatment with BIBF1000. The used dose of BIBF1000 was sufficient to inhibit the drug targets in heart tissue and, most importantly, to demonstrate the safety of BIBF1000 with respect to potential development of failure of the pressure overloaded RV.

A few previous studies addressed the effects of tyrosine kinase inhibitors (TKI)s on RV adaptation to pressure overload. Kojonazarov et al. (27), showed in monocrotaline treated and PAB rats that the multi kinase inhibitors Sunitinib (PDGFR, VEGFR and c-Kit inhibition) and Sorafenib (raf, VEGFR and PDGFR inhibition) reduced RV hypertrophy, fibrosis and vascular remodeling. Importantly, the degree of pressure overload in these studies was diminished after TKI treatment, while a load independent assessment of RV function was not made. As such firm conclusions pertaining to the effects of these TKIs on cardiac adaptation cannot be made. A
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decrease in RVSP after PAB is most likely explained by growth inhibition, as the
pressure gradient over a stenosis with a fixed diameter is determined by blood flow.
In the normal and well-adapted heart, cardiac output is strongly related to body
weight. In fact, we observed that treatment with 50 mg/kg BIBF1000 hampered body
weight gain which also decreased cardiac output, whereas CI was unchanged. This
finding indicates that our dosing strategy was sufficiently rigorous, and that the
absence of cardiac side effects was not due to under-dosing. Additionally, the finding
stresses the fact that a proper evaluation of RV function in a PAB study should
include load-independent parameters of RV function. Although no significant
differences in RVSP were observed between treated and non-treated PAB rats, it can
be speculated that the diminished RV hypertrophy and ANP expression after
BIBF1000 treatment was not a direct pharmacological effect, but rather due to a
relatively lower load due to the impaired body weight gain. Because we also show
that the EES/EA ratio (a load independent indicator of RV function) was unaffected
by BIBF1000 treatment, we can conclude that the drug does not adversely affect RV
function, even in the presence of pulmonary hypertension. Within the limits of this
animal model, in BIBF1000-treated PAB rats, a trend towards impaired capillary
growth (number of capillaries per cardiomyocyte; (n/cmc) was noted, which was in
line with a lower cross sectional area of cardiomyocytes. As a potential explanation,
the hampered body weight gain in BIBF1000-treated PAB rats might result in a
slightly reduced degree of right heart hypertrophy. As a result, fewer capillaries were
necessary to maintain a normal capillary density (per mm²). It is questionable if
extending the treatment period of BIBF1000 would have given a more definite answer
to the question whether BIBF1000 might hamper capillarization. However, the degree
of RV pressure overload in the PAB model is corresponding to the increased animal
body weight and will stop when the animal reaches its adult body weight. This
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means, practically, that the seen effect will not increase in magnitude when the
protocol would be extended to 2 months. Although this decrease was not sufficient
for RV worsening, monitoring RV function is warranted at higher dose concentrations
of BIBF1000 or when longer dosing periods are applied. In a potential confirmatory
clinical trial in patients with IPF and concomitant PH the determination of RV capillary
flow reserve would be the best prognostic measurement if RV deterioration is
suspected (42).

CONCLUSION:
Given the absence of differences in cardiac index, RV arterial coupling, connective
tissue disposition and capillary density, BIBF1000 treatment has no effects on the
pressure overloaded RV. We conclude that in this experimental model, concomitant
inhibition of VEGFR, FGFR and PDGFR does not hamper cardiac adaptation to
pressure overload.

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Disclosures
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REFERENCES


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Legends to the figures:

Figure 1: Inhibition of PDGF-receptor and FGF-receptor signaling by BIBF1000 in heart tissue. Expression of the phosphorylated PDGFRα/β (A) and the downstream signaling molecule of the FGFR pERK 1/2 (B) was assessed densitometrically from Western blots. Mean plus SEM, * = p<0.05, ** = p<0.01, *** = p<0.001, 3 animals per group.

Figure 2: Body weight gain in vehicle- and BIBF1000-treated groups of sham-operated (panel A) and Pulmonary Artery Banding (PAB)-operated (panel B) rats. Mean plus SEM, ** = p<0.01, *** = p<0.001. Number of animals per group; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.

Figure 3: Echocardiographic parameters in vehicle- and BIBF1000-treated groups of sham-operated and Pulmonary Artery Banding (PAB)-operated rats; Right Ventricular Wall Thickness (RVWT, panel A) and Right Ventricular End Diastolic Diameter (RVEDD, panel B), Tricuspid Annular Plane Systolic Excursion (TAPSE, panel C) and Cardiac Index (CI, panel D). Mean plus SEM, ** = p<0.01, *** = p<0.001. Number of animals per group; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.

Figure 4: Right Ventricular Systolic Pressure (RVSP, panel A), End Systolic Elastance (EES, panel B), Aterial Elastance (EA, panel C), ventriculo-arterial coupling; (EES/EA, panel D), End Diastolic Elastance (EED, panel E), Stroke Volume Index (SVI, panel F) and Left Ventricular Systolic Pressure (LVSP, panel G) in vehicle- and BIBF1000-treated animals of sham-operated and Pulmonary Artery...
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Banding (PAB)-operated rats. Mean plus SEM, *** = p<0.001. Number of animals per group for RVSP; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7. Number of animals per group for other experiments; sham vehicle 7, sham BIBF1000 6, PAB vehicle 7, PAB BIBF1000 7.

Figure 5: Hematocrit (panel A) and organ weights in vehicle- and BIBF1000-treated animals of sham-operated and Pulmonary Artery Banding (PAB)-operated rats. RV weight was corrected by tibia length regarding growth retardation (RV/tibia, panel B), as well the Left Ventricle ((LV+S)/tibia, panel C). RV weight divided by left ventricle plus septum weight based on the tibia corrected values (RV/(LV+S), panel D). Mean plus SEM, * = p<0.05, ** = p<0.01, *** = p<0.001. Number of animals per group for hematocrit; sham vehicle 7, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7. Number of animals per group for RV, LV+S and (RV/(LV+S)); sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.

Figure 6: Histological analyses in vehicle- and BIBF1000-treated animals of sham-operated and Pulmonary Artery Banding (PAB)-operated rats; Cross Sectional Area (CSA, panel A), capillaries (CD31+ cells per cardiomyocyte, panel B), capillaries (CD31+ cells per mm², panel C), collagen tissue (panel D) and Inflammatory density (CD45+ nuclei per mm², panel E). Mean plus SEM, * = p<0.05, *** = p<0.001. Number of animals per group; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.

Figure 7: ANP-protein expression measured by Western blot in vehicle- and BIBF1000-treated animals of sham-operated and Pulmonary Artery Banding (PAB)-
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operated rats. Mean plus SEM, * = p<0.05. Number of animals per group; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.

Figure 7: Expression of ERK (A) and Akt (B) and the phosphorylation (p-ERK and p-AKT) were measured by Western blot in heart tissue of vehicle- and BIBF1000-treated animals of sham-operated and Pulmonary Artery Banding (PAB)-operated rats. Mean plus SEM. Number of animals per group; sham vehicle 8, sham BIBF1000 8, PAB vehicle 8, PAB BIBF1000 7.
A

B

BW (grams)

-10  0  10  20  30  40

time (days)

sham - vehicle
sham - BIBF1000

PAB - vehicle
PAB - BIBF1000

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