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ORIGINAL ARTICLE

Combination Therapy of Pulmonary Arterial Hypertension with Vardenafil and Macitentan Assessed in a Human Ex Vivo Model

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Abstract

Purpose Treatment of pulmonary arterial hypertension (PAH) by vasodilator drug monotherapy is often limited in its effectiveness. Combination therapy may help to improve treatment and to reduce drug toxicity. This study assessed the combination of the endothelin receptor antagonist macitentan and the phosphodiesterase-5 inhibitor vardenafil in a human ex vivo model.

Methods Study patients did not suffer from PAH. Human pulmonary arteries (PA) and veins (PV) were harvested from resected pulmonary lobes. Contractile forces of blood vessel segments in the presence and absence of the vasodilator drugs macitentan, its main metabolite ACT-132577, and vardenafil were determined isometrically in an organ bath.

Results Macitentan 1E-7 M was sufficient to significantly abate endothelin-1-induced vasoconstriction in PA. A concentration of 1E-6 M was required for significant effects of macitentan on PV and of ACT-132577 on both vessel types. Combination of 1E-7 M macitentan and 1E-6 M vardenafil inhibited sequential constriction with endothelin-1 and norepinephrine of PA significantly more than either compound alone. Effects of 3E-7 M and 1E-6 M macitentan and effects of all doses of ACT-132577 were not further enhanced by 1E-6 M vardenafil.

Conclusions These data suggest that vasodilator effects of macitentan and vardenafil combined may surpass monotherapy in vivo if drug doses are adjusted properly. Vasodilation by the longer-acting metabolite ACT-132577 was not further enhanced by vardenafil.

Keywords Pulmonary arterial hypertension · Macitentan · Vardenafil · Combination therapy · Endothelin receptor antagonist · Phosphodiesterase-5 inhibitor

Introduction

Pulmonary arterial hypertension (PAH) is a progredient disease of the lung which is characterized by an elevated blood pressure on the arterial side of the pulmonary circulation resulting from pulmonary vascular disease [1]. While there is no cure available to date, current treatment strategies employ several vasodilator therapies which reduce the pulmonary artery pressure. Available medications target calcium channels, prostacyclin receptors, endothelin-1 receptors, and the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway [1, 2]. Some of these therapies additionally help to decelerate the progress of the disease by inhibiting vascular remodeling and vascular cell proliferation. The selection of suitable medications is based on the pathophysiology and on the severity of the disease. Randomized placebo-controlled trials have demonstrated a small but significant reduction of mortality and improvements of exercise capacity regardless of the vasodilator mechanism [3].

Current guidelines [4–6] and evidence-based treatment algorithms [7] suggest administration of calcium channel blockers in patients with a positive vasoreactivity testing response. Otherwise, or if the treatment goal cannot be achieved with these drugs, low- to intermediate-risk patients should

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receive monotherapy (one of endothelin receptor antagonists (ERAs), prostacyclin analogs, or phosphodiesterase-5 (PDE-5) inhibitors) or sequential combination therapy, i.e., addition of one or two drugs targeting different pathways later if needed. High-risk patients should receive initial combination therapy consisting of two drugs targeting different pathways, adding a third one if needed.

This underlines the importance of combination vasodilator therapies. Although medications have to be selected in each patient individually, direct measurement of the vasodilators' effects on blood vessel tension in a model system allows assessment of the utility of particular combinations of medications. We have reported an evaluation of combination therapies in an ex vivo system using human pulmonary vessels previously, using the ERA bosentan and various PDE-5 inhibitors. Results indicated beneficial effects of combinations with sildenafil [8] and with vardenafil but not with tadalafil [9]. The high potency of vardenafil in this model agrees with its potent inhibition of isolated PDE-5 [10]. Macitentan is an alternative dual ET_A/ET_B receptor antagonist with increased efficacy and safety compared with bosentan [11]. Macitentan does not require bioactivation. However, its main metabolite ACT-132577 has similar vasodilator properties as the parent compound and thus needs to be considered in an ex vivo model as well. The biological half-lives in humans of macitentan and ACT-132577 were determined as 17.5 h and 66 h, respectively [12]. Macitentan has been approved for PAH therapy using a 10 mg once-per-day regimen. Plasma levels of macitentan peaked at approx. $1.6 \cdot 10^{-7}$ M after a single 5-mg dose in healthy subjects, and at approx. $5.7 \cdot 10^{-7}$ M after a 25-mg dose [12]. Plasma levels of vardenafil peaked at approx. $4 \cdot 10^{-9}$ M after a single 20-mg dose; the half-life was measured as 4 to 5 h [13].

The purpose of the present study was to investigate the combination effects of macitentan and vardenafil on human pulmonary vessels in an established ex vivo system. In addition to the parent compound, its metabolite ACT-132577 was evaluated as well.

Materials and Methods

Study Design

Participants were recruited prospectively among patients who were scheduled to undergo elective pulmonary lobectomy in the Department of Thoracic Surgery, University of Regensburg Medical Center, Germany; in the Department of Thoracic Surgery, Hospital Barmherzige Brüder, Regensburg, Germany; or in the Department of Cardiothoracic and Vascular Surgery, University of Ulm Medical Center, Germany. Patients were eligible if they were at least 18 years of age, had no clinical signs of PAH, and were able to provide written informed consent. Exclusion criteria included known infectious diseases such as hepatitis and preoperative chemo- or radiotherapy. Pulmonary arteries and veins (internal diameter 5 to 10 mm) were excised from regions of the resected lobes that were not directly affected by the patient's disease. Care was taken not to distend the vessels or to damage the endothelium.

Analysis of Contractile Forces

Pulmonary vessels were cut transversally into rings of 2 mm segment length. These were mounted in an organ bath equipped with isometric force transducers (IOA 5300, FMI GmbH, Seeheim/Ober-Beerbach, Germany). The organ bath technique has been described previously [8, 14]. In brief, the baths were filled with Krebs-Henseleit buffer and gassed with carbogen (95% O_2, 5% CO_2), thus maintaining a pH of 7.4. Basal contractile forces were adjusted to 16 mN and 12 mN in pulmonary arteries (PA) and veins (PV), respectively. Stability of the baseline was assessed before and after a transient contraction in response to 150 mM KCl. Vessels were excluded if the maximum contraction in the absence of inhibitors failed to reach 4 mN, if spastic behavior was observed, and if the baseline was not stable.

Blood vessels harvested from the patients were assigned consecutively to one of the following experimental protocols.

Endothelin Receptor Antagonist Characterization

These experiments were performed on four rings of PA and four rings of PV taken from the same patient. Different doses of macitentan or of ACT132577 were added to equilibrated vessel rings. Control rings received an equivalent volume of solvent instead. After 15 min of incubation, endothelin-1 (ET-1) dose-response curves ranging from 10^{-11} M to 10^{-7} M were constructed by adding the next concentration as soon as the previous concentration had reached its maximum effect.

Combination Effects

Combination effects of ERA and vardenafil were established in experiments using eight rings of PA from a single patient. Two rings were assigned to each of the treatments: (a) control, receiving solvent only; (b) one of the ERA; (c) vardenafil; and (d) ERA and vardenafil combined. Substances were administered 15 min before a submaximal dose-response curve up to 10^{-8} M ET-1 was recorded, immediately followed by a norepinephrine (NE) dose-response curve running from 10^{-8} M to 10^{-5} M NE. The administration of two different vasoconstrictors was required to demonstrate combined vasodilator effects of ERA and PDE-5 inhibitors [8]. Drug concentrations were based on the abovementioned characterizations of ERA and on previously published effects of vardenafil [9].

Drugs and Reagents

Vardenafil was purchased from Chemos (Regenstauf, Germany). Norepinephrine was supplied by Aventis (Frankfurt/Main, Germany). ET-1 was obtained from Alexis (Läufelfingen, Switzerland). Macitentan and ACT-132577 were generous gifts from Actelion Pharmaceuticals Deutschland GmbH (Freiburg, Germany). All other reagents were of at least p.a. grade and were purchased from Sigma (Taufkirchen, Germany) and from VWR (Darmstadt, Germany). Stock solutions of vardenafil, macitentan, and ACT-132577 were prepared and, if necessary, further diluted in dimethyl sulfoxide.

Data Analysis and Statistics

Contractile forces are presented as changes from baseline. Effects of the receptor antagonists on baseline force were determined by calculating the difference of the forces at the beginning and at the end of the preincubation. Data are presented as median (interquartile range) with n indicating the number of patients. Box plots show median as well as first and third quartiles. The whiskers extend to the lowest and highest value up to 1.5 times the interquartile range below and above the first and the third quartile, respectively. Values beyond the whiskers indicate outliers. Statistics and regressions were computed with R [15] including its nlme and multcomp packages. Effects of substances on baseline forces were analyzed by the Wilcoxon signed-rank test. Dose-response curves were compared by mixed model analysis, using vasoconstrictor concentration and treatments as independent factors and patients as source of random effects. If both factors had significant effects, data were split along vasoconstrictor concentrations; these were tested for the significance of the factor treatment. Tukey's post-tests provided pairwise comparisons of the treatments. Effects or factors were assumed to be significant if the p value was less than 0.05. Half-maximal effective concentrations (EC_{50}) were determined by nonlinear least-squares fitting of the Hill equation.

Results

Study Patients

Vessels of 70 patients were assigned to the experimental protocols from January 2015 to June 2018. Demographic data of the 58 patients whose vessels met the criteria to be included into the analysis are presented in Table 1.

Table 1 Demographic data of the patients whose vessels met the inclusion criteria. Data indicate the number n and the percentage of participants except for age in years which is given as median (interquartile range)

Participants	
Included into analysis	58
Male gender	42 (72%)
Age	69.0 (62.3 to 73.0)
Surgery	
Upper lobe	36 (62%)
Middle lobe	1 (2%)
Lower lobe	16 (28%)
Upper + lower lobe	1 (2%)
Upper lobe + segmentectomy	1 (2%)
Pneumonectomy	3 (5%)
Left/right side	25 (43%)/33 (57%)
Indication for surgery / histology	
Bronchial carcinoma	57 (98%)
Carcinoid tumor	1 (2%)

Effects of Endothelin Receptor Antagonists

This series of experiments was used to establish (1) potential effects of the ERA on basal tone which may indicate endogenous ET-1 release as a nuisance factor and (2) suitable concentrations of the antagonists for combination experiments. Neither macitentan nor ACT-132577 significantly affected basal tones of PA and PV compared with the corresponding solvent controls ($p > 0.56$, $n = 6$–8). Figure 1 shows the data for 10^{-5} M ERA. The lower concentrations 10^{-6} M and 10^{-7} M were comparable (not shown). Both macitentan ($p < 0.0001$, Fig. 2) and ACT-132577 ($p < 0.0001$, Fig. 3) inhibited ET-1-induced vasoconstriction of pulmonary arteries

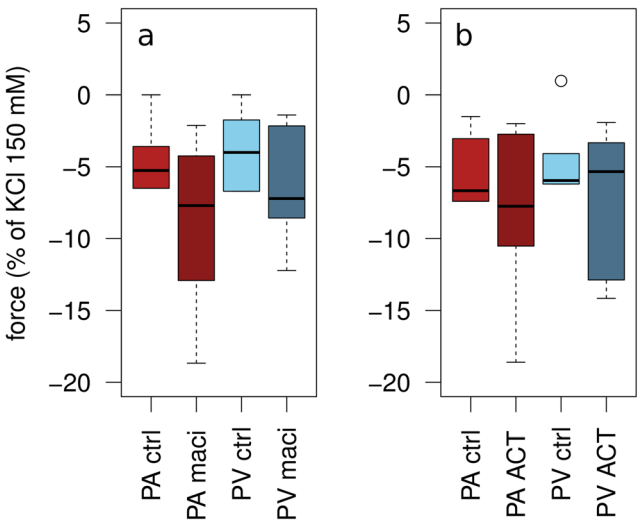

Fig. 1 Box plots of the changes of basal contractile forces after adding 10^{-5} M macitentan (maci, panel a) and 10^{-5} M ACT-132577 (ACT, panel b) and the corresponding solvent controls (ctrl) to human pulmonary arteries (PA) and veins (PV). Substance treatments did not differ significantly from their solvent controls. PA, macitentan $n = 6$; PV, macitentan $n = 9$; PA, ACT-132577 $n = 6$; PV, ACT-132577 $n = 7$. Image was created with R

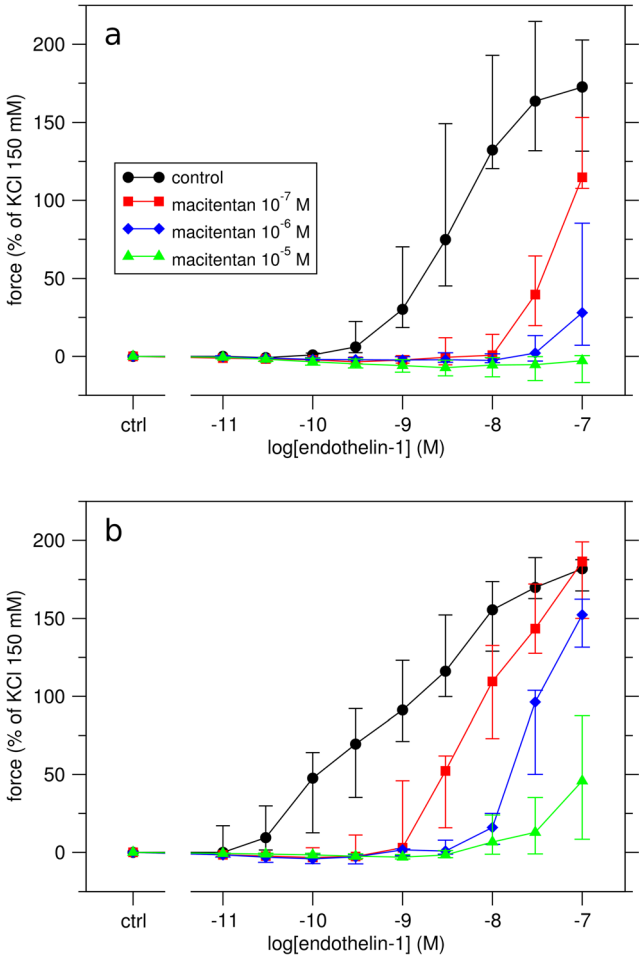

Fig. 2 Effect of macitentan (circles, no macitentan; squares, 10^{-7} M; diamonds, 10^{-6} M; triangles, 10^{-5} M) on endothelin-1 dose-response curves in pulmonary arteries (panel a, $n = 6$) and veins (panel b, $n = 9$). Force data are presented as median normalized to the responses to potassium chloride (KCl); lower and upper whiskers indicate 1st and 3rd quartiles, respectively. Image was created with Grace

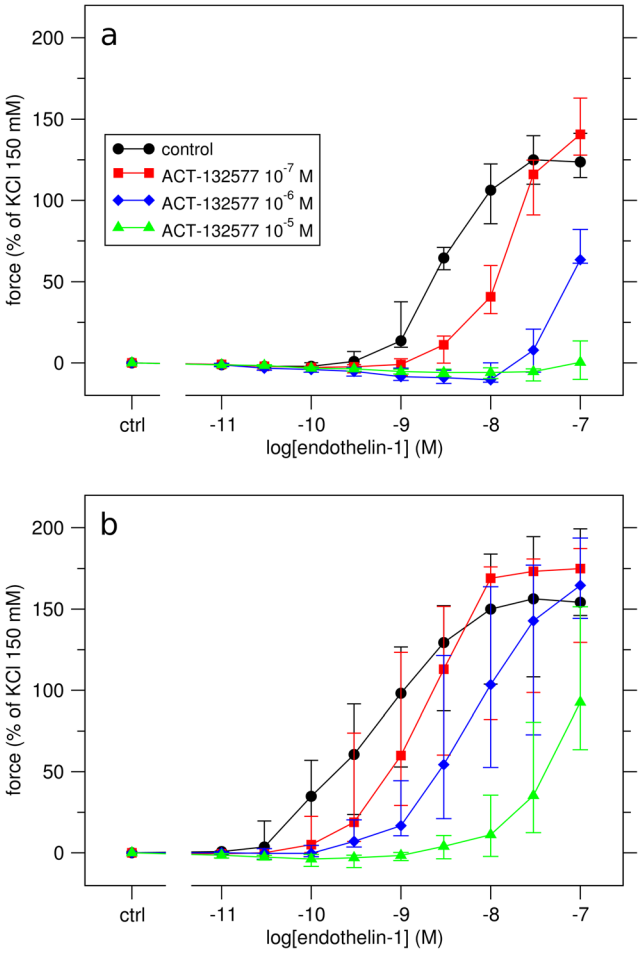

Fig. 3 Effect of ACT-132577 (circles, no ACT-132577; squares, 10^{-7} M; diamonds, 10^{-6} M; triangles, 10^{-5} M) on endothelin-1 dose-response curves in pulmonary arteries (panel a, $n = 6$) and veins (panel b, $n = 7$). Force data are presented as median normalized to the responses to potassium chloride (KCl); lower and upper whiskers indicate 1st and 3rd quartiles, respectively. Image was created with Grace

and veins in a dose-dependent fashion. Post-tests of PA data indicate that all macitentan doses differed from the control and from each other ($p < 0.02$), except for the comparison of 10^{-6} M and 10^{-5} M macitentan ($p = 0.054$). Inhibition in PV was less potent as 10^{-7} M macitentan did not significantly affect forces ($p = 0.475$). Higher concentrations differed from control ($p < 0.0001$), and 10^{-7} M differed from both higher concentrations ($p < 0.0001$). The apparent difference between 10^{-6} M and 10^{-5} M was not significant ($p = 0.419$).

ACT-132577 at 10^{-7} M did not differ significantly from the control PA values ($p = 0.475$), and the doses 10^{-6} M and 10^{-5} M did not elicit different responses ($p = 0.475$), with all other comparisons indicating significant differences ($p < 0.0001$). In PV, 10^{-7} M ACT-132577 did not significantly inhibit contraction ($p = 0.417$), whereas all other concentrations differed from control and from each other (all p values ≤ 0.048). Table 2 summarizes the EC_{50} values of the curves of Figs. 2 and 3.

Table 2 EC_{50} values (M) of endothelin-1 in the presence of macitentan and of ACT-132577. *nc* no convergence owing to insufficient curve coverage. PA, macitentan $n = 6$; PV, macitentan $n = 8$; PA, ACT-132577 $n = 6$; PV, ACT-132577 $n = 7$

Macitentan	Pulmonary artery	Pulmonary vein
Control	2.69×10^{-9}	8.26×10^{-10}
10^{-7} M	8.72×10^{-8}	7.81×10^{-9}
10^{-6} M	nc	3.30×10^{-8}
10^{-5} M	nc	3.30×10^{-8}
ACT-132577	**Pulmonary artery**	**Pulmonary vein**
Control	3.57×10^{-9}	9.06×10^{-10}
10^{-7} M	1.64×10^{-8}	1.58×10^{-9}
10^{-6} M	nc	5.09×10^{-9}
10^{-5} M	nc	5.09×10^{-9}

Combination Effects of Macitentan and Vardenafil

Three different concentrations of macitentan were used in combination experiments with 10^{-6} M vardenafil (Fig. 4). In the series with 10^{-8} M macitentan, the factor treatment significantly influenced contractile forces ($p < 0.0001$, $n = 7$). Vardenafil did not differ from control at any of the vasoconstrictor doses ($p > 0.636$). Macitentan showed a noticeable but incomplete inhibition of ET-1-induced contractions starting at 10^{-9} M ET-1 and up to 10^{-8} M ET-1 ($p \leq 0.047$). In contrast, the combination of macitentan and vardenafil differed from the control from 10^{-9} M ET-1 to the end of the NE concentration series ($p \leq 0.0079$). The combination elicited significantly stronger inhibition compared with both single inhibitors at $3 \cdot 10^{-7}$ M, 10^{-6} M, and 10^{-5} M NE ($p \leq 0.0425$), leaving 68% of the contractile force of the corresponding controls at 10^{-5} M NE.

In the presence of $3 \cdot 10^{-8}$ M macitentan, there was an incomplete but significant inhibition of ET-1-induced vasoconstriction which persisted to the end of the NE dose-response curve. Treatment had a significant effect on force starting at 10^{-9} M ET-1 ($p \leq 0.0019$, $n = 6$). The combination of macitentan and 10^{-6} M vardenafil did not differ from macitentan alone ($p \geq 0.877$), but from vardenafil ($p < 0.003$), and reached 72% of the control at 10^{-5} M NE.

In the series with 10^{-7} M macitentan, the factor treatment also had a significant effect on contractile forces ($p < 0.0001$, $n = 6$), with macitentan ($p \leq 0.0345$) and combination ($p \leq 0.0384$) treatments differing from controls starting from 10^{-9} M ET-1 to 10^{-6} M NE and to 10^{-5} M NE, respectively. Although the maximum contraction of 56% of controls by ET-1 and NE combined was even lower with 10^{-7} M macitentan compared with the lower doses, there was no additional effect of vardenafil ($p \geq 0.971$).

Combination Effects of ACT-132577 and Vardenafil

The combination experiments were repeated using macitentan's main metabolite ACT-132577 as ERA (Fig. 5). At the lowest dose, treatment did not significantly affect forces ($p = 0.155$, $n = 6$), whereas there were significant effects at $3 \cdot 10^{-8}$ M ($n = 9$) and 10^{-7} M ($n = 8$) ACT-132577 ($p < 0.0001$) on ET-1-induced but not on NE-induced contractions. The combination of $3 \cdot 10^{-8}$ M ACT-132577 with 10^{-6} M vardenafil differed from control at 10^{-10} M through 10^{-9} M ET-1 ($p \leq 0.049$). ACT-132577 alone differed from control at 10^{-9} M and $3 \cdot 10^{-9}$ M ET-1 ($p \leq 0.0305$), whereas vardenafil did not differ from control ($p > 0.122$).

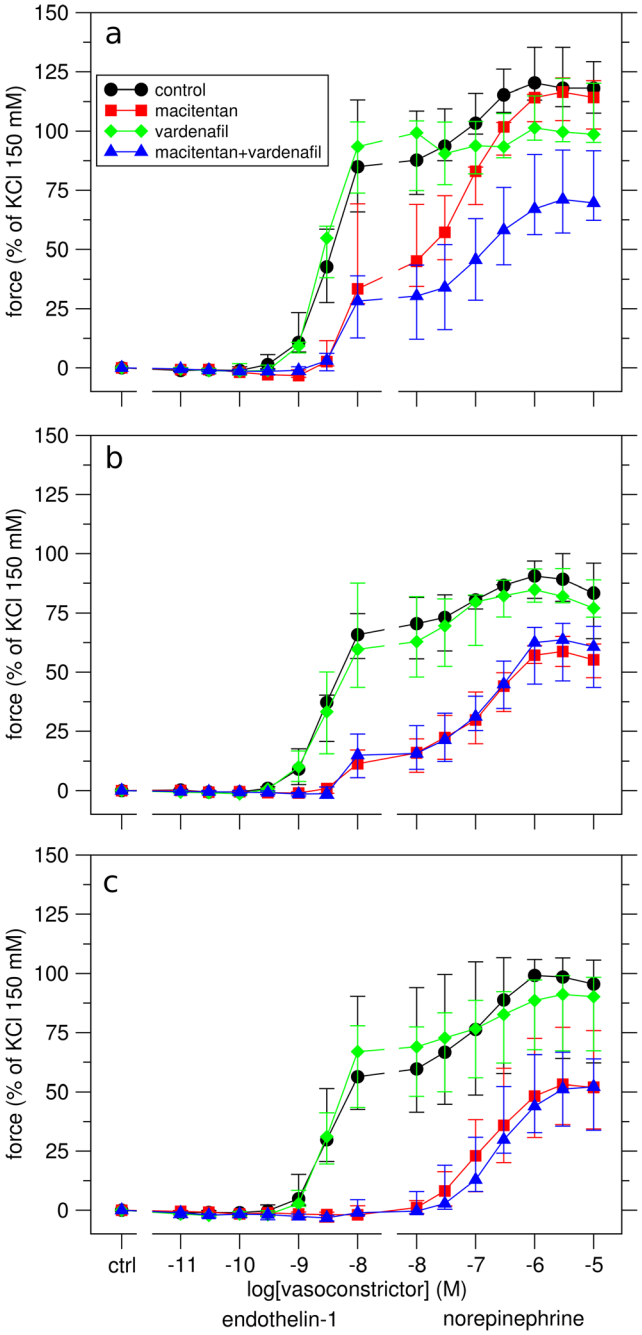

Fig. 4 Combination effects of macitentan (panel a, 10^{-8} M; b, $3 \cdot 10^{-8}$ M; c, 10^{-7} M) and vardenafil (10^{-6} M) on endothelin-1 and norepinephrine-induced vasoconstriction of pulmonary arteries. Force data are presented as median normalized to the responses to potassium chloride (KCl); lower and upper whiskers indicate 1st and 3rd quartiles, respectively. Image was created with Grace

At the highest dose of 10^{-7} M ACT-132577, treatment significantly affected forces at ET-1 doses of $3 \cdot 10^{-9}$ M through 10^{-8} M. ACT-132577 differed from control at $3 \cdot 10^{-10}$ M through 10^{-8} M ($p \leq 0.0178$), whereas ACT-132577 and vardenafil combined differ from control only at 10^{-9} M and $3 \cdot 10^{-9}$ M ET-1 ($p < 0.007$). There was no significant effect of vardenafil ($p \geq 0.8775$).

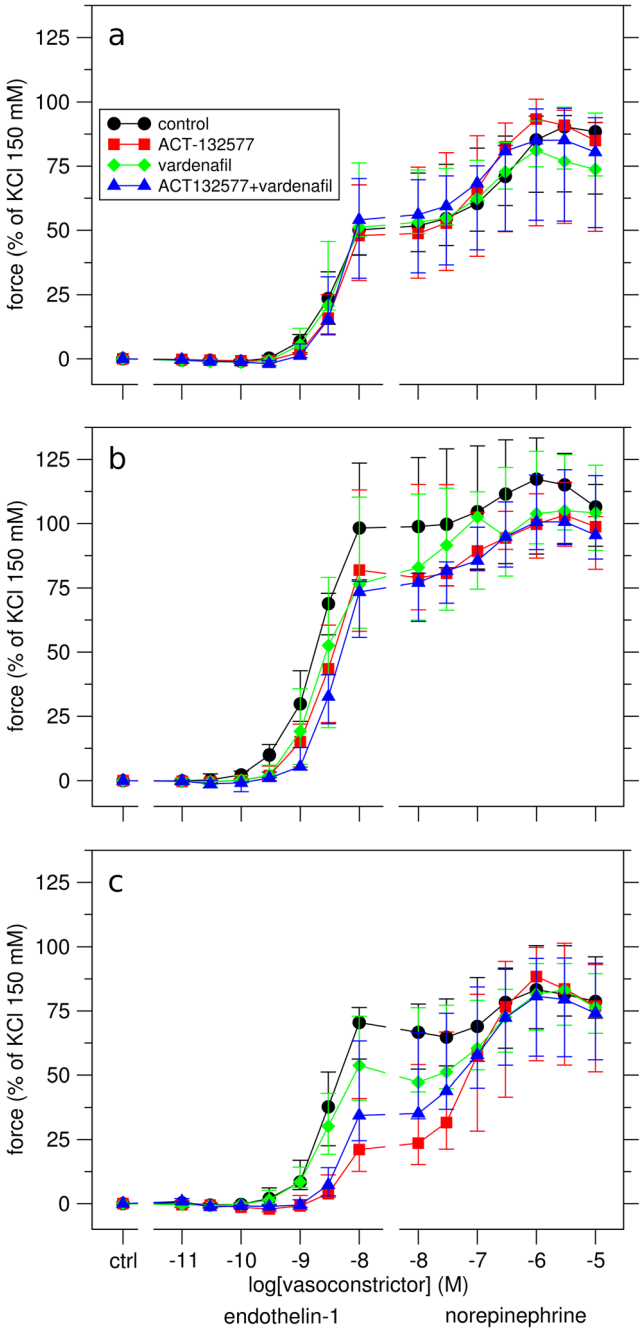

Fig. 5 Combination effects of ACT-132577 (panel a, 10^{-8} M; b, $3 \cdot 10^{-8}$ M; c, 10^{-7} M) and vardenafil (10^{-6} M) on endothelin-1 and norepinephrine-induced vasoconstriction of pulmonary arteries. Force data are presented as median normalized to the responses to potassium chloride (KCl); lower and upper whiskers indicate 1st and 3rd quartiles, respectively. Image was created with Grace

Discussion

Although there are approved medications for PAH targeting different signaling pathways, treatment of patients' symptoms and, more importantly, their influence on mortality is still considered unsatisfactory [6]. There is an ongoing search for additional drug targets which may replace or complement the existing ones. Besides drugs that affect cell proliferation, remodeling, and inflammation, there are also vasodilator targets which are not currently being exploited. These include the serotoninergic system and ion channels besides the established calcium channels [16]. For the time being, vasodilator combination therapy using established targets seems to provide reduced clinical worsening compared with monotherapy, and thus the best possible treatment of PAH patients [17]. The present study focused on the combination of ERA and PDE-5 inhibitors.

Since the discovery of endothelin-1 and of its receptors in the lung [18, 19], several orally active non-peptide receptor antagonists were developed. Bosentan is a mixed ET_A/ET_B receptor antagonist; it was the first ERA approved for the treatment of PAH in 2001. The compound showed beneficial effects on exercise capacity and on the Borg dyspnea index of PAH patients in early single-center [20] and multi-center [21] randomized placebo-controlled trials. First-line bosentan was also shown to improve survival [22]. Functional effects of bosentan have been investigated in isolated rat aortas and rabbit superior mesenteric arteries [23] as well as in isolated human pulmonary vessels [8]. Sitaxentan, a highly selective ET_A antagonist [24], was approved in some markets in 2006 but withdrawn before a final FDA approval in 2010 owing to concerns about liver damage [25]. Ambrisentan, another ET_A antagonist, was approved in 2007 [26]. Macitentan was developed as a successor of bosentan with an improved liver safety profile and prolonged receptor binding [27]. The drug entered the market in 2013. A review of randomized controlled studies of all approved ERAs concluded that these drugs are an appropriate treatment for PAH patients, although larger studies and longer follow-up times are desirable [28].

The vasodilator action of phosphodiesterase inhibitors is mediated by the increased concentration of intracellular cGMP in vascular smooth muscle cells. Inhibitors specific for PDE-5 are particularly useful as this isoenzyme has a suitable distribution between pulmonary and systemic circulation [29]. Sildenafil was approved for the treatment of PAH in 2005 after a double-blind randomized placebo-controlled trial had shown improved exercise capacity and hemodynamics [30] when added to the patients' conventional therapy. Tadalafil was approved for the same indication in 2009, providing the benefit of a once-daily dosage regimen with similar clinical benefits as sildenafil in a study where a part of the PAH patients used bosentan concomitantly [31]. Vardenafil is currently only approved for the treatment of erectile dysfunction, although it has been shown to improve exercise capacity and to reduce pulmonary arterial pressure as a monotherapy in a double-blind randomized placebo-controlled study [32]. Vardenafil appears to be less specific for the pulmonary circulation compared with sildenafil and tadalafil [33].

Measurement of the effects of monotherapies on isolated blood vessel tensions is an established technique. We have analyzed the dose-response relationships of vardenafil in human pulmonary arteries and veins previously [9], concluding that it is a more potent PDE-5 inhibitor than sildenafil and tadalafil. We observed that PDE-5 inhibitors require higher concentrations to elicit vasodilation in the organ bath model compared with the plasma levels which are effective therapeutically. This also explains why vardenafil as the strongest of the PDE-5 inhibitors was preferred to tadalafil in the present study in spite of the longer duration of action in vivo of the latter. The present study provided dose-response relationships of macitentan and of its metabolite ACT-132577. Both substances did not significantly alter basal tone of pulmonary arteries and veins, indicating that there is no appreciable contribution of endogenous endothelin to the vessel tone. Also, paradox vasoconstrictor effects did not occur. Macitentan significantly inhibited ET-1-induced contractions of both pulmonary arteries and veins. A dose of 10^{-7} M was sufficient to significantly decrease tension of pulmonary arteries, increasing EC_{50} approx. 30-fold. For comparison, the same dose of bosentan shifted the curve approx. 7-fold in our previous study [8]. Both 10^{-6} M and 10^{-5} M macitentan abolished contractions almost completely in the tested ET-1 concentration range. Potency in pulmonary veins was lower by approximately one order of magnitude. ACT-132577 appeared to be less potent in both pulmonary arteries and veins, as it did not elicit significant responses at the lowest dose. However, this reduced potency must be set off against a longer half-life in vivo, which may allow the metabolite to contribute substantially to macitentan's overall effects. The tested concentrations of macitentan and of ACT-132577 were comparable to the plasma levels observed in humans [12].

The demonstration of the effects of combination therapies in an organ bath requires a more elaborate approach. To demonstrate ERA effects, ET-1 has to be present. We found it necessary to add an NE dose-response curve on top of a submaximal contraction with ET-1 in order to measure effects related to an elevated cGMP concentration, the main effect of PDE-5 inhibitors [8, 9]. Previous data had indicated that 10^{-5} M vardenafil attenuated contraction in these combined dose-response curves to a degree that made it impossible to analyze additional ERA effects. Vardenafil at 10^{-6} M and macitentan at the lowest dose of 10^{-7} M caused a significantly better inhibition of vasoconstriction than either substance alone. In contrast, higher doses of macitentan again were too potent on their own to see any additional effects of vardenafil. This indicates that a careful titration of vardenafil and macitentan doses may be desirable in vivo to obtain the best possible effect with the lowest drug doses, although this is currently beyond approved dosing regimens.

In contrast to macitentan, its metabolite ACT-132577 inhibited ET-1-induced contractions significantly only at $3 \cdot 10^{-8}$ M and 10^{-7} M. In both cases, the inhibitor effects were detectable only during the ET-1 dose-response curve, whereas there were no effects during NE administration. Therefore, under none of the tested conditions did the combination of ACT-132577 and vardenafil differ from both vardenafil and ACT-132577 alone. The different combination effects of macitentan and vardenafil vs. ACT-132577 and vardenafil may thus result in intricate pharmacokinetics of the drug combination. This may be exacerbated by the high interindividual variability of vardenafil pharmacokinetics [34].

Several clinical trials have tried to assess the benefits of combination therapy over monotherapy in PAH treatment. A recent meta-analysis identified a total of seven randomized placebo-controlled trials that compared ERA and PDE-5 inhibitors combined versus monotherapy as a primary goal or by subgroup analyses [17]. The meta-analysis concluded that the studies showed an overall positive effect of combination therapy vs. monotherapy in terms of clinical worsening and of exercise capacity. Most of these trials combined bosentan or ambrisentan with a PDE-5 inhibitor, whereas the SERAPHIN trial seems to be the only trial so far combining macitentan with PDE-5 inhibitors. The study protocol allowed oral or inhalative baseline therapy to be continued throughout the trial that compared two doses of macitentan to placebo. The trial showed significant reductions of morbidity and mortality by macitentan over control [35] irrespective of the use of baseline therapies. At the time of this writing, there were no other registered trials comparing dual therapy with macitentan and PDE-5 inhibitors to monotherapy. The only trial to date that measured the effects of initial (as opposed to sequential) combination therapy compared with monotherapy used ambrisentan and tadalafil as ERA and PDE-5 inhibitor, respectively, and demonstrated a lower risk of a composite of death, hospitalization, disease progression, and treatment failure in patients who received combination therapy [36].

The present study used an ex-vivo model and thus suffers from specific limitations. First, drug interactions that are related to extravascular drug metabolism do not play a role in isolated blood vessels. Second, the study used a predefined vardenafil concentration and varied ERA concentrations only. Interindividual differences in responses to both substance classes may require a more individual dosing of both drugs to obtain best results; this approach would require larger amounts of pulmonary vessels from a single patient than what is usually available. Third, the concentrations of PDE-5 inhibitors required to elicit vasodilations are higher than the clinically observed plasma levels; this may require further refinements of the organ bath model. Finally, the modeling of the combination effects using two classes of vasoconstrictors, ET-1 and NE, is also likely to cover only a part of the relevant vasoconstrictor signals that play a role in vivo.

Conclusions

Macitentan is a potent inhibitor of ET-1-induced vasoconstriction in isolated human pulmonary blood vessels and displays a limited specificity for arterial over venous vessels. Its metabolite ACT-132577 is a less potent inhibitor in both vessel types. Selected concentrations demonstrate an additive effect of a combination therapy of macitentan and vardenafil whereas none of the tested combinations showed a benefit of combination therapy over ACT-132577 alone. These ex vivo results indicate that the combined vasodilator effects of macitentan and vardenafil may be useful to lower pulmonary blood pressure in vivo. Prospective randomized clinical trials are required to assess the benefits of this type of combination therapy.

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Author Contribution Statement MH, HSH, and MR conceived and designed the study. CG, LS, HSH, and MR recruited patients and harvested blood vessels. MH, SG, LE, and MS conducted experiments. MH analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committees of the University of Regensburg (file no. 11-101-0133) and of the University of Ulm (file no. 384/13) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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