Plasma Drug Concentrations in Patients with Pulmonary Arterial Hypertension on Combination Treatment

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Keywords

Pulmonary arterial hypertension · Combination therapy · Sildenafil · Tadalafil · Bosentan · Ambrisentan · Macitentan

Abstract

Background: Combination therapy with the phosphodiesterase type 5 inhibitors (PDE-5i) sildenafil or tadalafil and the endothelin receptor antagonists (ERA) bosentan, ambrisentan, or macitentan may cause mutual pharmacokinetic interactions in patients with pulmonary arterial hypertension (PAH). **Objective:** The objective of this study was to analyze plasma drug concentrations in PAH patients receiving different combination treatments. **Methods:** PAH patients receiving a stable combination treatment with ERA and PDE-5i with targeted dosage for at least 1 month were routinely assessed, including clinical parameters and plasma drug concentrations. Concentrations were normalized considering dose and time from last medication intake and presented as multiples of the expected mean (MoM) of the respective monotherapies. **Results:** A total of 125 PAH patients (84 female, 41 male, 57% idiopathic/heritable) were included. Sildenafil and tadalafil concentrations were lowest in combina-

tion with bosentan (MoM 0.44 ± 0.42 , 95% confidence interval [CI] 0.30–0.57, and MoM 0.89 ± 0.53 , 95% CI 0.50–1.28, respectively) compared to the combination with ambrisentan (MoM 1.3 ± 0.97 , 95% CI 0.86–1.73, and MoM 1.67 ± 0.63 , 95% CI 1.40–1.94, respectively) and macitentan (MoM 1.16 ± 0.87 , 95% CI 0.86–1.46, and MoM 1.59 ± 0.99 , 95% CI 0.80–2.38, respectively). The combination of sildenafil and bosentan led to more than twice the expected bosentan concentrations in 53.8%. Patients switching from sildenafil-bosentan to macitentan showed a significant increase in sildenafil concentrations ($p < 0.001$). **Conclusions:** Only the combination with macitentan or ambrisentan led to targeted mean PDE-5i plasma concentrations and should therefore be preferred to combination with bosentan. Sildenafil-bosentan showed the strongest interaction, with low sildenafil and high bosentan concentrations. The study was not powered to analyze whether lower PDE-5i concentrations cause unsatisfying clinical response. However, plasma concentrations within a targeted range are desirable and may become of increasing importance.

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Introduction

Within the last 15 years, 11 targeted drugs for the medical treatment of pulmonary arterial hypertension (PAH) have been approved in many countries [1], and the possibility to combine 2 or more classes of drugs has become increasingly relevant. Three separate signaling pathways of the disease can be addressed by specific drugs such as prostanoids, soluble guanylate cyclase stimulators, endothelin receptor antagonists (ERA; bosentan, macitentan, and ambrisentan), and phosphodiesterase type 5 inhibitors (PDE-5i; sildenafil and tadalafil). With increasing use of combination therapy, drug-drug interactions have to be considered.

In the recently published COMPASS-2 trial, combination therapy with bosentan and sildenafil compared with sildenafil and placebo did not show an improvement of the composite primary endpoint time to clinical worsening [2]. Insufficient sildenafil drug plasma concentrations due to pharmacokinetic interactions between bosentan and sildenafil were discussed as a possible reason for the lack of efficacy, but were not tested [2]. Similar results were obtained for the combination of tadalafil and bosentan in the PHIRST study, with a less pronounced effect in patients pretreated with bosentan compared to treatment-naïve patients, which may possibly be attributed to a decrease in tadalafil plasma concentrations when coadministered with bosentan [3].

The combination of bosentan and sildenafil is one of the most common combination treatments for PAH in adults worldwide [4]. Drug-drug interactions of this medication combination resulting in different plasma concentrations would be of significant clinical impact. In healthy subjects [5, 6] and in PAH patients [7, 8], it has already been shown that bosentan significantly reduces the maximum plasma concentration of sildenafil by approximately 50% and that of tadalafil by up to 26%. Conversely, sildenafil inhibits hepatic uptake of bosentan by inhibition of organic anion-transporting polypeptides [9]. Therefore, bosentan plasma concentration increased by ~40% when bosentan was coadministered with sildenafil in healthy subjects [5], but was not affected when combined with the weaker organic anion-transporting polypeptide inhibitor tadalafil [6, 10]. Due to this known interaction, the package inserts of both bosentan and sildenafil inform that the combination of the 2 agents leads to an increase in sildenafil and a decrease in bosentan, but is not thought to require dose adjustments. In healthy subjects, it has been shown that the interaction between sildenafil and bosentan becomes stronger over time, with a more pronounced decrease in sildenafil and an

increase in bosentan concentrations after 16 days, compared to 6 or 10 days of combination treatment [5]. This might be due to the time of the bosentan-mediated enzyme induction of cytochrome P450 (CYP) isozymes and the synthesis of new enzymes, which are responsible for the degradation of most lipophilic drugs such as sildenafil and bosentan [11–13]. However, medication interactions of combination treatment for at least 1 month have not yet been investigated in a larger cohort of PAH patients. In contrast, in dedicated studies in healthy volunteers, an interaction of ambrisentan with sildenafil or tadalafil was ruled out [14, 15], whereas the coadministration of macitentan with sildenafil mildly increased sildenafil peak concentrations by 26% [16].

Most pharmacokinetic interaction studies are conducted in healthy volunteers and typically assess a short-term combination therapy early after or even before reaching steady-state conditions. Therefore, the importance of pharmacokinetic interactions of the long-term combination of PDE-5i and ERA in clinical practice remains unclear. In other areas, the observed extent of interaction was smaller than predicted from studies in healthy volunteers (e.g., direct oral anticoagulants [17]).

The objective of this study was to analyze plasma drug concentrations in PAH patients receiving various combination treatments with a stable dose of different ERA and PDE-5i for at least 1 month. Furthermore, we aimed to assess changes in ERA and PDE-5i plasma concentrations after switching to a different ERA due to clinical worsening or lack of clinical improvement. We also analyzed the potential impact of comorbidity (connective tissue disease [CTD], renal impairment with estimated glomerular filtration rate <60 mL/min/1.73 m²) and coadministration of imatinib on PAH drug exposure.

Imatinib is an antiproliferative agent that was developed for the treatment of chronic myeloid leukemia. As this agent also shows inhibitory effects on the platelet-derived growth factor and c-KIT signaling, it was suggested that it may be efficacious in PAH, but it was not approved due to side effects and serious adverse events [18]. Patients who participated in the phase III trial often continued treatment with imatinib due to good clinical response and positive effects on quality of life [19].

Subjects and Methods

Study Population and Design

Patients with invasively confirmed PAH who received targeted combination treatment were consecutively included from March 2015 on by clinical routine examinations and assessment of medi-

cation plasma concentrations in the Center for Pulmonary Hypertension, Thorax Clinic at the University Hospital Heidelberg. Patients with former routine assessments of medication plasma concentrations were asked for their permission to retrospectively include these results and corresponding clinical data. PAH-targeted treatment had to be on a stable dose for at least 1 month before plasma concentration measurement. Patients entered the study on their individually established dose of PAH medication, and neither patients nor clinicians knew the drug plasma concentrations. All decisions about drug dosing were uninfluenced by the concentration measurements.

Clinical routine assessments comprised medical history, concomitant medication, concomitant diseases, demography, vital signs, electrocardiography, echocardiography, routine blood tests including NT-proBNP, and 6-min walking distance as described previously [20, 21]. The data of the most recent right heart catheterization were used for hemodynamics.

In remnants of routine blood samples that were taken before the next scheduled combination therapy intake, the plasma concentrations of sildenafil, tadalafil, bosentan, and ambrisentan were measured using validated, sensitive mass spectrometry (LC-MS/MS) methods as previously described [22]. At the time of analysis, the measurement of macitentan concentrations was not methodologically established. Dosing schedule and last intake were documented for later interpretation of the results. Potentially interacting comedication with targeted PAH drugs was checked using the AiDKlinik software [23].

Elimination organ dysfunctions such as hepatic and renal impairment were diagnosed by a specialist physician (nephrologist, gastroenterologist) and presented among other criteria of respective organ dysfunction with creatinine >1.1 mg/dL or glomerular filtration rate <60 mL/min/ 1.73 m² (renal impairment), or increased gamma-glutamyl transferase ≥ 60 U/L for male patients and >40 U/L for female patients (hepatic impairment). The study had a positive unrestricted vote of the responsible Ethics Committee of the Medical Faculty of Heidelberg University (ID S-605/2014), and all patients gave their written informed consent for participation in the study. The study was registered at clinicaltrials.gov (NCT02484807).

Normalization of Measurements: Multiple of the Expected Mean

Individual plasma concentrations were interpreted in relation to the expected mean medication concentrations at the respective sampling time point in the dosing interval. The expected mean concentration ranges refer to data extracted from published plasma concentration-time profiles measured during monotherapy with sildenafil [24, 25], tadalafil [26], bosentan [27–29], and ambrisentan [30] and served as comparative values. To calculate dose- and time-specific expected mean values, dose proportionality was assumed for each agent according to the literature [26, 31–33]. Each individually measured drug concentration was set in proportion to the expected mean concentration and expressed as a multiple of the expected mean (MoM), with values <1 denoting lower and values >1 higher values than the expected mean. For better orientation and interpretation, target ranges of plasma drug concentrations were defined. The commonly used ranges to prove bioequivalence of generic drugs are 80–125% regarding the maximum concentration or area under the curve of medication concentrations. The bioequivalence ranges derive from the FDA Guidance (Guidance for Industry. Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommen-

dations) as an initial cutoff for an inhibitor/inductor. To define ranges with marked difference from expected values, we introduced a conservative definition for the targeted medication concentrations representing a more distinct inhibition/induction at one point in time. The ranges were expanded to 0.5–2.0 times the expected mean plasma concentration, corresponding to half (lower limit = 0.5) or twice (upper limit = 2.0) the expected mean plasma concentrations in order to achieve a conservative assumption of targeted medication concentrations.

Statistical Analysis

The analyses were performed by 2 statisticians (N. Benjamin, C. Fischer). Data are presented as mean $\pm$ standard deviation or sample size and respective percentage. Additionally, 95% confidence intervals (CIs) are given for effect size estimation. Due to the exploratory nature of this study, no sample size analysis was conducted in advance of the trial.

Differences in MoM between groups were analyzed by ANOVA. Individual comparisons between single combination treatment groups and analysis of influencing factors were performed by the Wilcoxon rank-sum test. Plasma concentrations before and after transition were compared by the Wilcoxon signed-rank test.

A sensitivity analysis excluding patients with known influencing factors such as concomitant disease or known interacting comedications was performed to rule out the effect of known interactions. Furthermore, a potential influence of individual drug doses on drug concentrations was analyzed by ANOVA with the drug combination as fixed factor. The association of clinical data and medication concentrations was analyzed by Pearson correlation analysis using clinical data from 6-min walking distance, echocardiographic parameters, and blood gas analysis.

All tests were 2-sided and p values <0.05 were considered statistically significant. All analyses were carried out with IBM SPSS V22 (IBM Corp., Armonk, NY, USA).

Results

The clinical characteristics of the study cohort are given in Table 1. Plasma drug concentrations were assessed in 125 patients; 67% were female, 57% had idiopathic/heritable PAH, 10.4% had congenital heart disease-associated PAH, 21% had CTD-associated PAH, and 11.6% had PAH with comorbidities (pre- and postcapillary components of pulmonary hypertension due to additional comorbidity, atypical PAH). Mean pulmonary arterial pressure was 47 ± 15 mm Hg, and 43.4% were in WHO functional class (WHO-FC) II, 53.3% in WHO-FC III, and 3.3% in WHO-FC IV. They were receiving targeted PAH combination treatment for a median of 14 months (ERA, range 1–194 months) and 13 months (PDE-5i, range 2–194 months). All plasma concentrations were measured at trough before the next planned medication intake. Renal insufficiency was reported in 44 (35.2%) patients, hepatic insufficiency based on elevated gamma-

Table 1. Clinical characteristics of the study cohort at baseline at first ERA/PDE-5i measurement

	Bosentan-sildenafil	Ambrisentan-sildenafil	Macitentan-sildenafil	Bosentan-tadalafil	Ambrisentan-tadalafil	Macitentan-tadalafil
Total number	39	19	33	7	21	6
Demography						
Age, years	56±16	61±19	65±13	56±19	63±13	64±21
Height, cm	166±10	165±9	169±9	166±11	168±6	172±7
Weight, kg	74±22	75±17	81±19	73±16	77±17	72±9
Female sex	29 (74.4%)	15 (78.9%)	19 (57.6%)	5 (71.4%)	12 (57.1%)	4 (66.7%)
Body mass index	27.0±7.5	27.4±5.6	28.1±5.8	26.5±5.5	27.1±4.5	24.7±3.1
Medication						
Daily ERA dose, mg/kg	240±35	8±3	10±0	250±0	9±3	10±0
Daily PDE-5i dose, mg/kg	178±89	147±90	73±26	43±8	37±10	27±10
Diagnosis						
IPAH	20	9	17	3	12	1
HPAH	5	2	1	1	0	0
CHD-associated PAH	5	1	5	2	0	0
CTD-associated PAH	4	5	4	0	9	4
Atypical PAH	5	2	6	1	0	1
Hemodynamics						
Mean PAP (RHC), mm Hg	53±15	49±16	41±14	44±14	45±15	36±11
Cardiac output, L/min	4.9±1.5	4.7±1.4	4.8±1.7	4.5±1.2	4.8±1.9	5.1±1.1
PVR, dynes × s × cm ⁻⁵	693±287	649±400	503±317	501±339	669±401	411±335
Systolic PAP (Echo), mm Hg	64±20	59±23	48±19	60±29	43±14	40±15
Exercise capacity						
Six-minute walking distance, m	399±120	371±110	392±115	408±113	397±97	395±100
Laboratory						
NT-proBNP, ng/mL	2,023±3,304	2,756±6,813	594±782	1,287±1,247	2,073±5,892	5,019±9,516
PDE-5i concentration, MoM	0.44±0.42	1.30±0.97	1.16±0.87	0.89±0.53	1.67±0.63	1.59±0.99
ERA concentration, MoM	3.54±3.24	1.81±0.82	not assessable	2.18±1.23	1.53±0.84	not assessable

Values are presented as mean ± standard deviation or *n* (%). CHD, congenital heart disease; CTD, connective tissue disease; Echo, echocardiography; ERA, endothelin receptor antagonist; HPAH, heritable pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; MoM, multiple of the expected mean; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAH, pulmonary arterial hypertension; PAP, pulmonary arterial pressure; PDE-5i, phosphodiesterase type 5 inhibitor; PVR, pulmonary vascular resistance; RHC, right heart catheterization.

glutamyl transferase in 4 (3.2%). None of the patients had significantly elevated transaminase levels. Echocardiography, 6-min walking distance, and NT-proBNP levels were done on the day of the routine visit, while hemodynamic parameters measured by right heart catheterization were obtained from the most recent invasive hemodynamics assessment (Table 1).

Medication Plasma Concentrations in Different Combinations of ERA and PDE-5i

Plasma concentrations of PDE-5i significantly differed between the different combination treatments with ERA

(ERA-sildenafil, $p < 0.001$; ERA-tadalafil, $p = 0.042$; Fig. 1). Sildenafil and tadalafil concentrations were lowest in combination with bosentan (MoM 0.44 ± 0.42 , 95% CI 0.30–0.57, and MoM 0.89 ± 0.53 , 95% CI 0.50–1.28, respectively) compared to the combination with ambrisentan (MoM 1.3 ± 0.97 , 95% CI 0.86–1.73, and MoM 1.67 ± 0.63 , 95% CI 1.40–1.94, respectively) and macitentan (MoM 1.16 ± 0.87 , 95% CI 0.86–1.46, and MoM 1.59 ± 0.99 , 95% CI 0.80–2.38, respectively; Fig. 1).

Bosentan plasma concentrations compared to ambrisentan concentrations were significantly higher in combination with sildenafil, while ERA concentrations

Fig. 1. Boxplots showing the distribution of phosphodiesterase type 5 inhibitor (PDE-5i) concentrations expressed as MoM in different combination treatments. Plasma concentrations significantly differed between the different endothelin receptor antagonists (ERA) for both combination with sildenafil (ANOVA, $p < 0.001$) and tadalafil (ANOVA, $p = 0.042$). PDE-5i concentrations were lowest in combination with bosentan. Boxplots represent the median as horizontal line, the lower and upper quartile as box, 1.5× interquartile range as whiskers, and outliers as dots and asterisks.

Fig. 2. Boxplots showing the distribution of endothelin receptor antagonist (ERA) concentrations expressed as MoM in different combination treatments with phosphodiesterase type 5 inhibitor. ERA concentrations significantly differed for sildenafil (ANOVA, $p = 0.026$), but not for tadalafil (ANOVA, $p = 0.129$). Boxplots represent the median as horizontal line, the lower and upper quartile as box, 1.5× interquartile range as whiskers, and outliers as dots and asterisks.

did not differ when combined with tadalafil (ERA-sildenafil, ANOVA $p = 0.026$; ERA-tadalafil, ANOVA $p = 0.129$; Fig. 2).

Ranges of Drug Concentrations Using Multiples of the Expected Mean

The frequencies of plasma concentration ranges of PDE-5i for each combination treatment are given in Table 2 and for sildenafil-bosentan in Figure 3. In 28 out of 39 patients (71.8%, 95% CI 57.7–85.9%) receiving combination treatment with sildenafil-bosentan, sildenafil concentrations were lower than half of the expected value, corresponding to a <0.5 MoM, while individual concentrations for bosentan showed a >2.0 MoM in 53.8% of cases ($n = 21/39$, 95% CI 38.2–69.5%; Fig. 3). Thus, most of the patients receiving long-term combination treatment of sildenafil and bosentan (sildenafil 30 ± 38 months; bosentan 51 ± 43 months) presented with PDE-5i concentrations which were very low (<0.5 MoM; Fig. 3). In many of these patients only trace amounts of sildenafil were detected in plasma. In combination with other ERA, sildenafil concentrations were mostly in the range between 0.5 and 2.0 MoM (ambrisentan, $n = 14/19$, 73.7%, 95% CI 53.9–93.4%; macitentan, $n = 22/33$, 66.7%, 95% CI 50.6–82.8%). In these combinations, targeted sildenafil plasma concentra-

Fig. 3. In patients receiving sildenafil-bosentan, sildenafil concentrations were below half of the expected value in 71.8% of cases, whereas bosentan concentrations showed values above double the expected value in >50% of cases. The MoM range between 0.5 (half of the expected value) and 2.0 (twice the expected value) is indicated as a shaded area.

tions were measured in almost all patients (Table 2; Fig. 4). However, even in the combination with other ERA, a few patients had levels <0.5 MoM (sildenafil-ambrisentan, $n = 3/19$, 15.8%; macitentan-sildenafil, $n = 6/33$, 18.2%; Table 2).

MoM of tadalafil were mainly in the target range of 0.5–2.0 MoM for combination with bosentan ($n = 6/7$, 85.7%, 95% CI 59.8–100%), except for 1 patient with MoM <0.5, ambrisentan ($n = 17/21$, 81.0%, 95% CI 64.2–97.7%), and macitentan ($n = 3/5$, 50.0%, 95% CI 10–90%; Table 2; Fig. 4).

Factors Influencing Drug Concentrations

The analysis of factors potentially influencing drug exposure included sex, elimination organ dysfunction, CTD, and comedication such as imatinib, treprostinil, or dipyrrone. Furthermore, the relationship between ERA and PDE-5i dose/kg body weight and drug exposure was analyzed.

Sex and renal and hepatic impairment did not affect plasma concentrations of ERA or PDE-5i in our study cohort. Patients with CTD ($n = 26$) had significantly lower drug concentrations than patients without CTD within each combination subgroup (ANOVA with medication combination as fixed factor, $p = 0.04$; data not shown). Twenty-three patients (18.4%) were on potentially interacting comedication; 5 patients (4.0%) received imatinib, 17 (13.6%) treprostinil (6 subcutaneously, 11 intravenously), and 1 (0.8%) dipyrrone. Imatinib was associated with significantly higher ERA plasma concentrations (MoM 4.72 ± 1.73 , 95% CI 3.05–6.44, vs. MoM 2.48 ± 2.45 , 95% CI 1.95–3.01; ANOVA, $p = 0.013$). Treprostinil did not show a significant influence on medication plasma concentrations (ANOVA, $p = 0.428$). Due to the small sample size, analysis for dipyrrone with only 1 patient receiving sildenafil-ambrisentan was not performed.

Sildenafil doses/kg body weight varied depending on the combination partner. Significantly lower dosages/kg

Fig. 4. Plot showing the medication concentrations for phosphodiesterase type 5 inhibitors (PDE-5i) with respect to the applied dosages/kg body weight. Significantly higher sildenafil dosages/kg body weight were given in patients receiving bosentan. The dosage/kg body weight did not significantly influence the MoM plasma concentrations, with comparable MoM of sildenafil in the combination of sildenafil-bosentan irrespective of the dosage/kg and different MoM for comparable dosages/kg for combination with other endothelin receptor antagonists (ERA).

Table 2. Frequencies of PDE-5i plasma concentration ranges in different combination treatments

	PDE-5i concentration, MoM					
	<0.5		0.5–2		>2	
Bosentan-sildenafil	28	71.8% (57.7–85.9%)	10	25.6% (11.9–39.4%)	1	2.6% (0–7.5%)
Ambrisentan-sildenafil	3	15.8% (0–32.1%)	14	73.7% (53.9–93.5%)	2	10.5% (0–24.3%)
Macitentan-sildenafil	6	18.2% (5.0–31.3%)	22	66.7% (50.6–82.8%)	5	15.2% (2.9–27.4%)
Bosentan-tadalafil	1	14.3% (0–40.2%)	6	85.7% (59.8–100%)	0	0%
Ambrisentan-tadalafil	0	0%	17	81.0% (64.2–97.7%)	4	19.0% (2.3–35.8%)
Macitentan-tadalafil	1	16.7% (0–46.5%)	3	50.0% (10.0–90.0%)	2	33.3% (0–71.1%)

Values are presented as *n* and percentage (95% confidence interval). MoM, multiple of the expected mean; PDE-5i, phosphodiesterase type 5 inhibitor.

body weight were applied in patients receiving sildenafil-macitentan (0.96 ± 0.43 mg/kg, 95% CI 0.82–1.11) compared to sildenafil-bosentan (2.57 ± 1.54 mg/kg, 95% CI 2.09–3.06; $p < 0.001$) or sildenafil-ambrisentan (1.98 ± 1.22 mg/kg, 95% CI 1.43–2.53; $p < 0.001$). However, the individual dose/kg was not significantly associated with PDE-5i concentrations (ANOVA, $p = 0.19$; Fig. 4). Sensitivity analyses excluding patients with known interacting conditions such as concomitant disease or concomitant medication (imatinib) led to the same results for dis-

tribution of medication concentrations and influencing factors.

No significant correlations were found between clinical data and MoM for ERA or PDE-5i.

Effect of Switching ERA on Sildenafil Exposure

In 20 patients receiving sildenafil-bosentan combination therapy, clinically indicated switching of ERA treatment to macitentan was performed. Reasons for change in medication were unsatisfactory long-term response

Fig. 5. Change of endothelin receptor antagonist treatment led to a significant increase in sildenafil concentrations ($p < 0.001$). The MoM range between 0.5 (half of expected value) and 2.0 (twice the expected value) is indicated as a shaded area.

($n = 10$), subjective dissatisfaction with exercise capacity (treatment optimization; $n = 4$), clinical worsening ($n = 5$), and adverse gastrointestinal side effects ($n = 1$). Medication switching was performed independent from drug plasma concentrations. At the time of switching, patients and clinicians did not know the results of medication concentrations.

After switching to macitentan (median, 3 months; range, 1–12 months), sildenafil concentrations showed a significant increase after transition compared to baseline values during bosentan treatment (MoM 0.42 ± 0.32 , 95% CI 0.28–0.56, vs. MoM 1.59 ± 1.46 , 95% CI 0.94–2.25; $p < 0.001$; Fig. 5). Before switching, 14 patients (74%) had sildenafil concentrations <0.5 MoM as opposed to only 3 patients (16%) thereafter. Patients who changed treatment remained clinically stable and showed a significant decrease in NT-proBNP ($2,204 \pm 3,823$ ng/mL, 95% CI

$529\text{--}3,880$, vs. $1,181 \pm 2,899$ ng/mL, 95% CI $-197\text{--}2,560$, $p = 0.003$; Table 3).

Discussion

This is the first study investigating the frequency and magnitude of drug-drug interactions and influencing factors in a large group of PAH patients on long-term combination treatment with PDE-5i and ERA. The most crucial substantial interaction was found in the combination of sildenafil-bosentan, with significantly lower concentrations of sildenafil and higher concentrations of bosentan. The study showed that $>70\%$ of our PAH patients receiving sildenafil-bosentan combination treatment for more than 1 month revealed insufficient sildenafil plasma concentrations, which were most likely associated with a

Table 3. Clinical data before and after medication switch from bosentan to macitentan

Characteristic	Baseline	Follow-up	<i>p</i> value
Sample size	20	19	
Clinical data			
WHO functional class			
II	6 (33.3%)	4 (26.7%)	ns
III	12 (66.7%)	11 (73.3%)	
Oxygen saturation at rest, %	92.2±4	93±4	ns
NT-proBNP, ng/mL	2,204±3,823	1,181±2,899	0.003
Echocardiography			
Systolic pulmonary arterial pressure, mm Hg	61±21	60±20	ns
Tricuspid annular plane systolic excursion, mm Hg	21.9±5.4	21.5±5.9	ns
Right ventricular function			
Good	3 (16.7%)	2 (11.1%)	
Slightly impaired	1 (5.6%)	1 (5.6%)	
Moderately impaired	7 (38.9%)	10 (55.6%)	ns
Severely impaired	7 (38.9%)	5 (27.8%)	
Drug concentration			
Sildenafil level, MoM	0.42±0.32	1.59±1.46	<0.001
ERA level, MoM	3.75±3.62	not assessable	

Values are presented as mean ± standard deviation or *n* (%). ERA, endothelin receptor antagonist; MoM, multiple of the expected mean; ns, not significant; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

reduced clinical effect. After transition of bosentan to macitentan, sildenafil concentrations significantly increased into the targeted range or above in >70% of the patients. This increase of sildenafil plasma concentrations was associated with a significant 46% decrease in NT-proBNP levels during follow-up. However, also in the combinations of other ERA and PDE-5i, low PDE-5i concentrations occurred in 1 patient each for tadalafil-macitentan and tadalafil-bosentan (14–18%), but not in the combination treatment of tadalafil-ambrisentan. Thus, drug-drug interactions may significantly influence plasma concentrations and may be clinically relevant, especially in case of an insufficient clinical response to therapy.

Interaction between PDE-5i and ERA

Our findings are in line with previous studies in healthy volunteers [5] or PAH patients [7, 8] reporting a significant interaction between sildenafil and bosentan. Sildenafil plasma concentrations decreased by 44% when co-administered with bosentan and increased in the presence of imatinib [8], a potent inhibitor of CYP3A [34].

The duration of combination treatment seems to play an important role for drug interactions because pretreated patients had more pronounced interactions than pa-

tients with newly initiated targeted combination treatment [5, 35]. Furthermore, it has been shown that higher dosages of bosentan are more likely to cause abnormal hepatic function [36]. Thus, the side effects of bosentan may even be more pronounced over time, with increasing interaction when coadministered with sildenafil.

Clinical Impact of Plasma Concentrations of Targeted ERA/PDE-5i

Drug interactions can have a clinical impact if the victim drug has a narrow therapeutic index and its plasma concentrations reach low (<0.5 MoM) or very high concentrations (>2 MoM). However, there are no larger-scaled studies yet systematically assessing the clinical impact of different plasma concentrations. Hatano et al. [35] showed in 8 patients with PAH that sildenafil as add-on treatment to bosentan acutely lowers sildenafil concentrations in the blood and results in a less pronounced effect on pulmonary vascular resistance, but a comparable effect on cardiac index compared to monotherapy. In contrast, in our cohort on chronic treatment, changes in clinical parameters before and after switching were not statistically significant, except for a significant decrease in NT-proBNP.

Our study was not designed and powered to detect clinical effects after a change of ERA treatment. Even though we did not find strong correlations with clinical data, the significant increase in sildenafil concentrations after switching of ERA from bosentan to macitentan is desirable. The results indicate that transition of an ERA might be an option in patients with unsatisfactory long-term clinical response and an alternative to treatment escalation by initiation of a new class of PAH-targeted drugs. In case series, change of treatment due to side effects or unsatisfactory clinical response from bosentan to ambrisentan [37] and sildenafil to tadalafil [38] seemed to be beneficial. In a case report with 7 subjects, sildenafil-ambrisentan treatment showed higher medication concentrations for sildenafil than combination of sildenafil-bosentan and was associated with a higher 6-min walking distance [39].

In our cohort, combination treatment with sildenafil-ambrisentan also showed plasma concentrations within the target range. Therefore, we expect that a switch to ambrisentan instead of macitentan might also be beneficial and would lead to reduction of unfavorable medication interactions. In addition, it should be mentioned that also a transition from sildenafil to tadalafil might be an option considered in case of low clinical response, in particular since the AMBITION study [40] published in 2015 showed advantageous effects on clinical outcome for combination of ambrisentan and tadalafil. Further investigations are needed to detect the effect of PDE-5i change on clinical parameters.

It is not known whether higher plasma concentrations lead to more side effects or are linked to better efficacy. Higher dosages have however shown a more pronounced improvement of hemodynamics in sildenafil [41] and a higher impact on exercise capacity and clinical worsening for tadalafil [3]. As treatment with bosentan has to be thoroughly monitored due to potential liver injuries and showed dose-dependent increases in serum aminotransferase abnormalities [36], drug interactions increasing bosentan concentrations have to be carefully checked [33]. Interestingly, we could not detect elevated transaminase levels in combination with increased bosentan concentrations >2.0 MoM. However, individual dosages should be adjusted according to clinical data and reported side effects of the patient. Further studies are needed to address the relevance of high plasma concentrations of both ERA and PDE-5i.

In patients with CTD, significantly lower drug concentrations were detected throughout the different combination therapies. This finding might be a sign of malabsorp-

tion, which is a common issue in patients with CTD [42]. Further studies are needed to assess the frequency and clinical impact of lower medication concentrations in CTD.

Pharmacokinetic Data

Since bosentan is not only metabolized via CYP3A4 and CYP2C9 [9], but also induces CYP3A4 itself, bosentan is prone to cause pharmacokinetic interactions with other CYP substrates [6, 11, 29, 43]. Since the metabolism of sildenafil and tadalafil predominantly depends on CYP3A4 activity [12, 13, 44], reduced plasma concentrations were observed when coadministered with bosentan but not with ambrisentan, which lacks CYP-inducing effects [45]. In a study investigating the pharmacokinetics of sildenafil-bosentan in healthy subjects, the pharmacokinetic data showed lower concentrations for sildenafil after 16 days compared to 6 or 10 days of combination treatment [5]. At the same time, sildenafil increases bosentan concentrations, leading to an even more pronounced interaction between the 2 agents. This counter-reaction is only known for the combination of sildenafil and bosentan and may also be one reason for higher sildenafil levels in combination with other ERA.

The higher sildenafil doses/kg that were found in patients receiving sildenafil-bosentan in our study cohort may have developed due to a decreasing clinical response after several months, when the interaction between the 2 agents was supposedly more pronounced, leading to a clinically indicated increase in sildenafil dosage. In comparison to sildenafil-macitentan, PDE-5i dosages in sildenafil-ambrisentan were also higher, which might also indicate an adaptation of sildenafil over time due to a subjective feeling of decreasing clinical response. As we did not investigate the development of plasma concentrations over time, we may however not draw conclusions about the causality of higher dosages in this treatment group.

In our study, elimination organ dysfunction did not lead to a significant difference in medication concentrations. This may however be influenced by the small sample size of patients with renal or hepatic insufficiency. Larger studies are needed to assess whether elimination organ dysfunction has an impact on medication concentrations in clinical practice.

The finding that administration of imatinib was associated with significantly higher ERA plasma concentrations raises the question whether the clinical impact of imatinib relies on its own intrinsic effect or is based on increased ERA concentrations. This question has also been addressed by Renard et al. [8], who investigated the

pharmacokinetic interactions between bosentan, sildenafil, and imatinib, and those findings were in line with ours. They reported that clinical effects did not depend on the level of ERA concentrations between the imatinib group and the placebo group, and concluded that the effect of imatinib could not be caused by only higher ERA concentrations, but was rather due to imatinib itself. In our study cohort, we did not investigate patients on imatinib monotherapy, and therefore a final conclusion cannot be drawn.

Limitations

The calculation of MoM is a helpful tool supporting the interpretation of individual drug plasma concentrations. However, there are currently no established therapeutic plasma concentration ranges, and data are mainly based on studies including healthy subjects on monotherapy. Based on the results of the SUPER trial [41], dose and thus presumably also concentration changes critically determine the clinical response. We cannot exclude that values <0.5 may also have a positive treatment effect, though a medication plasma concentration of less than half of the expected value is certainly not desirable.

Except for the values before and after transition of a small subgroup, we did not investigate the development of drug concentrations over time.

Conclusion

This is the first study investigating the frequency and magnitude of medication interactions and influencing factors in various combinations of PAH-targeted treatment in clinical practice in a larger cohort. The combination of sildenafil and bosentan showed the strongest interaction with low sildenafil and high bosentan concen-

trations. After change of the ERA treatment, sildenafil concentrations showed a significant increase. If clinically indicated, change of ERA treatment might therefore be considered instead of initiation of a triple combination therapy. Further studies are needed to investigate the impact of low drug plasma concentrations, the effect of plasma concentration changes over time, and the causality of influencing factors.

Acknowledgment

This study was supported by research grants from Actelion Pharmaceuticals Germany GmbH and GlaxoSmithKline.

Financial Disclosure and Conflicts of Interest

N. Benjamin received speaker honoraria from Actelion and Bayer. E. Grünig received speaker honoraria and consultancy fees from Actelion, Bayer, GSK, United Therapeutics, and Gilead. W.E. Haefeli received speaker honoraria or consultancy fees from Actelion, Aspen, AstraZeneca, Bayer, Berlin Chemie, BMS, Boehringer Ingelheim, Daiichi-Sankyo, Grünenthal, GSK, and Novartis. H. Klose received honoraria and consultancy fees from Actelion, GSK, Pfizer, Bayer, MSD, United Therapeutics, and OMT. S. Harutyunova, B. Egenlauf, J. Ohnesorge, C. Fischer, Y. Enderle, A. Huppertz, and J. Burhenne report no conflict of interest.

Author Contributions

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