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Changes in plasma levels of asymmetric dimethylarginine, symmetric dimethylarginine, and arginine after a single dose of vardenafil in patients with pulmonary hypertension

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ABSTRACT

Objective: We investigated whether vardenafil, a phosphodiesterase-5 inhibitor, alters plasma levels of asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and arginine.
Patients and methods: ADMA, SDMA, and arginine were measured (0–540 min) in 12 patients with pulmonary hypertension after a single oral dose of vardenafil. Invasive hemodynamic data were collected at baseline and after 60 min.
Results: A reduction in ADMA was observed at 30 and 45 min with a median change of −11.1% ($P = 0.021$) and −12.5% ($P = 0.002$). SDMA decreased with a median −5.3% change ($P = 0.032$) at 45 min. An increase in arginine, median 40.3% ($P = 0.002$), 45.0% ($P = 0.010$), and 77.1% ($P = 0.008$) was observed at 120, 300, and 540 min respectively. An increase in the arginine/ADMA ratio, median 11.7% ($P = 0.012$), 32.5% ($P = 0.003$), 26.5% ($P = 0.021$), 33% ($P = 0.007$), 48.5% ($P = 0.007$), and 63.1% ($P = 0.008$) was observed at 15, 45, 60, 120, 300, and 540 min respectively. There was a positive correlation between vardenafil exposure and the percent change in the arginine/ADMA ratio from baseline to 540 min ($r = 0.80$; $P = 0.01$). A correlation between baseline mean right atrial pressure (mRAP) and baseline ADMA ($r = 0.65$; $P = 0.023$), and baseline SDMA ($r = 0.61$; $P = 0.035$) was observed. A correlation between the baseline arginine/ADMA ratio and baseline cardiac output (CO) ($r = 0.59$; $P = 0.045$) and baseline cardiac index (CI) ($r = 0.61$; $P = 0.036$) was observed. Baseline arginine/ADMA ratio correlated with baseline mRAP ($r = -0.79$; $P = 0.002$). A correlation between change (0–60 min) in CI and change in arginine ($r = 0.77$; $P = 0.003$) as well as change in the arginine/ADMA ratio ($r = 0.61$; $P = 0.037$) was observed.
Conclusions: Vardenafil induced changes in ADMA, SDMA, arginine, and the arginine/ADMA ratio in patients with PH. An increase in arginine and the arginine/ADMA ratio was associated with improvement in CI.

1. Introduction

Pulmonary hypertension (PH) defined by right heart catheterization (RHC) as a mean pulmonary artery pressure (mPAP) equal to or above 25 mm Hg at rest, is a pathophysiological state associated with a number of clinical conditions and diseases leading to progressive rise in pulmonary vascular resistance (PVR) and right ventricular failure [1,2].

Nitric oxide (NO) is a potent pulmonary artery vasodilator and altered NO production has been implicated in the development of PH [3–6]. In the vasculature, nitric oxide (NO) is synthesized in the endothelium from L-arginine by the action of NO synthase (NOS) [7].

Asymmetric dimethylarginine (ADMA), an endogenous guanidino-substituted analogue of L-arginine, is synthesized by the methylation of protein arginine residues by the enzyme protein arginine methyltransferase 1 (PRMT1) [8]. ADMA competes with L-arginine for NOS and inhibits NO formation [9]. The accumulation of endogenous ADMA has been described in multiple disorders where NOS dysfunction has been implicated [10]. Elevated ADMA levels have been reported in patients with several different categories and subcategories of PH; idiopathic pulmonary arterial hypertension (PAH), PAH associated with congenital heart disease, chronic thromboembolic pulmonary hypertension (CTEPH), PAH associated with systemic sclerosis (SSc), and PAH associated with chronic hemolytic anemia [11–17]. The clinical

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significance of elevated circulating levels of ADMA in patients with PH has been demonstrated. Increased levels of ADMA have been associated with poor exercise capacity [15], unfavorable pulmonary hemodynamics and worse outcome [12,14].

Phosphodiesterase-5 (PDE5) inhibitors represent a class of drugs that enhance the NO mediated pulmonary vasodilatation by promoting the accumulation of cyclic guanosine monophosphate (cGMP) [18]. Among the three available PDE5 inhibitors sildenafil, tadalafil, and vardenafil, currently only sildenafil and tadalafil have gained regulatory approval for the treatment of PAH. However, vardenafil has been demonstrated to be effective and well tolerated in patients with PAH in a randomized, double-blind, placebo-controlled study [19], and in patients with different forms of PH including PAH in uncontrolled case reports and in a long term open label study [20–23]. We have previously demonstrated that a single oral dose of vardenafil has a rapid and significant effect on several cardiopulmonary hemodynamic variables in patients with PH, with reduction in mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance (PVR), and an increase in cardiac output (CO) and cardiac index (CI) [24]. Furthermore, as the main finding, it was demonstrated that the acute changes in mPAP and PVR correlated with the plasma vardenafil drug concentration [24]. In the present study, our primary aim, was to investigate whether administration of a single oral dose of vardenafil induces changes in circulating levels of ADMA in patients with PH.

To gain insight into the potential of vardenafil to exert effects on other metabolic pathways involved in the production and regulation of NO, we also measured plasma levels of arginine and symmetric dimethylarginine (SDMA) and calculated the arginine/ADMA ratio.

2. Material and methods

2.1. Patients

The current study comprises 12 patients with PH of different etiologies (see Table 1), representing World Health Organization (WHO) groups I–IV according to the latest updated clinical classification of PH (Nice, 2013), that were enrolled in the study between 2006 and 2007 [1]. The patient group consisted of females ($n = 8$) and males ($n = 4$), 29–85 years of age (median 54.5), in WHO PH functional class II or III. All patients were receiving at least one of the following therapies (diuretics, β-adrenoceptor blocker, ACE inhibitor, warfarin, angiotensin receptor blocker, digoxin, or Ca^{2+} antagonist). Four patients with a previous diagnosis of PAH were treated with a PH-specific medication (three with bosentan and one with sildenafil). The other eight patients were diagnosed with PH or PAH at the time of enrolment after confirmation at RHC. The inclusion criteria were PH patients with a resting mPAP ≥ 25 mm Hg measured by RHC and an age over 18 years. No patient met the exclusion criteria (severe liver dysfunction [Child–Pugh class C], or hypotension [<90/50 mm Hg]). Diagnostic procedures preceding patient recruitment included echocardiography, chest X-ray, lung function test, measurement of the distance walked in 6 min (6MWD) and in selected cases tomographic scan of the lung and pulmonary angiography. Patients were hospitalized 1 day before and 1 day after the RHC, and the indication for RHC was either hemodynamic follow-up of known PAH ($n = 4$) or for diagnostic confirmation of suspected PAH/PH ($n = 8$). All patients were fasting 24 h before the procedure, thus no medication was given prior to the RHC.

2.2. Study design

The study was an open-label trial performed at the regional PAH Center at Uppsala University Hospital. The hemodynamic and pharmacokinetic results have been presented elsewhere [24,25]. The current study includes 12 of these 16 patients from which baseline plasma data were collected for analysis of arginine, ADMA, and SDMA. Each patient received one single oral dose of either 5 mg ($n = 1$), 10 mg ($n = 1$) or 20 mg ($n = 10$) vardenafil (Levitra®, Bayer Schering Pharma) depending on age and liver function (see Table 1). During RHC hemodynamic and mixed venous oxygen saturation measurements were performed at baseline and 15, 30, 45 and 60 min after vardenafil administration. The RHC procedure and measurements have been described previously [24]. Blood samples for determination of arginine, ADMA, SDMA and vardenafil plasma concentrations were collected at baseline and 15, 30, 45, 60, 120, 300, and 540 min after peroral administration of vardenafil. Blood samples were collected into EDTA-tubes (BD Diagnostics) and were processed immediately in a thermostatic (20 °C) centrifuge (Jouan BR 3.11) for 10 min at 3000 rev min^{-1}. Plasma was separated and stored at −20 °C until analysis. Approval for the study was obtained from a local ethics review committee and conducted in accordance with Good Clinical Practice guidelines and the Helsinki Declaration. Written informed consent was obtained from all patients.

2.3. Pharmacokinetic data

The area under the plasma concentration-time curve (AUC) for vardenafil from time of dosing to 540 min was calculated using the trapezoidal rule. The dose and body weight normalized values (AUC_{norm}) were derived by dividing by dose per kg body weight (see Table 1).

2.4. Bioanalytical method

Blood samples for chemical analysis were taken at baseline during RHC and post RHC up to 540 min. Determination of plasma vardenafil

Table 1
Baseline demographic and clinical characteristics and information on administered vardenafil dose and dose and body weight normalized area under the plasma concentration–time curve values for vardenafil from time 0 to 540 min.

Patient number	Age (years)	Gender	Weight (kg)	Body surface area (m^2)	Clinical classification of PH (WHO groups I–IV)	WHO PH functional class	6MWD (m)	Dose of vardenafil (mg)	AUC_{norm} (μL^{-1} h kg mg^{-1})
1	50	female	53	1.5	PH due to left heart disease (II)	III	350	5	710
2	60	female	73	1.8	PH due to left heart disease (II)	II	585	20	239
3	29	female	57	1.6	Idiopathic PAH (I)	II	658	20	18
4	77	male	102	2.2	PH due to left heart disease (II)	III	NA	20	527
5	38	female	49	1.5	Idiopathic PAH (I)	III	300	20	304
6	35	male	70	1.9	PH due to left heart disease (II)	III	422	20	524
7	45	female	69	1.8	PAH associated with connective tissue disease (systemic sclerosis) (I)	III	347	20	83
8	85	female	78	1.8	CTEPH (IV)	II	251	10	244
9	76	male	69	1.9	PH due to lung disease (III)	III	211	20	258
10	44	male	90	2.2	Idiopathic PAH (I)	II	570	20	NA
11	72	female	65	1.7	PAH associated with connective tissue disease (systemic sclerosis) (I)	III	180	20	NA
12	59	female	63	1.7	Idiopathic PAH (I)	III	300	20	NA