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Research paper

Sildenafil reduces signs of oxidative stress in pulmonary arterial hypertension: Evaluation by fatty acid composition, level of hydroxynonenal and heart rate variability

Khrystyna Semen [a,1], Olha Yelisyeyeva [b,*], Iwona Jarocka-Karpowicz [c], Danylo Kaminskyy [d], Lyubomyr Solovey [e], Elzbieta Skrzydlewska [c], Ostap Yavorskyi [a]

[a] Department of Propedeutics of Internal Medicine #2, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv, Ukraine
[b] Department of Histology, Cytology and Embryology, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv, Ukraine
[c] Department of Analytical Chemistry, Medical University of Bialystok, Jana Kilinskego 1, 15089 Bialystok, Poland
[d] Department of Pharmaceutical, Organic, and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv, Ukraine
[e] Lviv Regional Clinical Hospital, Chernigivska 7, 79010 Lviv, Ukraine

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a rare multifactorial disease with an unfavorable prognosis. Sildenafil therapy can improve functional capacity and pulmonary hemodynamics in PAH patients. Nowadays, it is increasingly recognized that the effects of sildenafil are pleiotropic and may also involve changes of the pro-/antioxidant balance, lipid peroxidation and autonomic control. In present study we aimed to assess the effects of sildenafil on the fatty acids (FAs) status, level of hydroxynonenal (HNE) and heart rate variability (HRV) in PAH patients. Patients with PAH were characterized by an increase in HNE and changes in the FAs composition with elevation of linoleic, oleic, docosahexanoic acids in phospholipids as well as reduced HRV with sympathetic predominance. Sildenafil therapy improved exercise capacity and pulmonary hemodynamics and reduced NT-proBNP level in PAH. Antioxidant and anti-inflammatory effects of sildenafil were noted from the significant lowering of HNE level and reduction of the phopholipid derived oleic, linoleic, docosahexanoic, docosapentanoic FAs. That was also associated with some improvement of HRV on account of the activation of the neurohumoral regulatory component. Incomplete recovery of the functional metabolic disorders in PAH patients may be assumed from the persistent increase in free FAs, reduced HRV with the sympathetic predominance in the spectral structure after treatment comparing to control group. The possibilities to improve PAH treatment efficacy through mild stimulation of free radical reactions and formation of hormetic reaction in the context of improved NO signaling are discussed.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare, progressive disease characterized by sustained pulmonary arterial vasoconstriction and vascular remodeling ultimately leading to right ventricular failure and death. The pathophysiology of PAH in complex and involves abnormal proliferation of the vascular smooth muscle and endothelial cells, infiltration by the inflammatory cells, and fibrosis of the vascular elements [20]. Multiple factors including endothelial dysfunction with reduced NO bioavailability, increased production of reactive oxygen species (ROS), impaired synthesis of prostaglandin with PGI_2 deficiency and excessive production of various mediators (angiotensin II, endotelin-1, etc.) play role in the pathologic remodeling of the pulmonary vasculature [1]. Recently, the action of the inflammatory, procoagulant, antiapoptotic, and autoimmune mediators, as well as cell–cell and cell–matrix interactions have been recognized as

Abbreviations: DHA, docosahexanoic acid; EPA, eicosapentanoic acid; FA, fatty acids; FC, functional class; HF, high frequency; HNE, hydroxynonenal; HRV, heart rate variability; LA, linoleic acid; LF, low frequency; 6MWT, 6 min walk test; NOS, nitric oxide synthase; NT-proBNP–N, terminal pro-brain natriuretic peptide; OS, oxidative stress; PAH, pulmonary arterial hypertension; PDE, phosphodiesterase; PKA, protein kinase A; PKG, protein kinase G; pNN50, percentage of differences between adjacent normal RR intervals exceeding 50 ms; RMSSD, the square root of the mean squared differences of successive RR interval; ROS, reactive oxygen species; SDNN, standard deviation of normal RR intervals; TP, total power; VLF, very low frequency

* Corresponding author.
E-mail addresses: khrystyna_semen@yahoo.com (K. Semen), yelisol@gmail.com (O. Yelisyeyeva).
[1] Department of Propedeutics of Internal Medicine #2 Danylo Halystky Lviv National Medical University; 69, Pekarska street, 79010, Lviv, Ukraine. Tel.: +380 676702554; fax: +380 322603277

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important contributors to disease progression [66]. Despite the progress in understanding PAH and major advances in its treatment the prognosis of the disease still remains unfavorable with the mortality rate of 10–15% per year and median survival of approximately 7 years [5] underscoring the need for more effective therapeutic options.

Currently approved PAH specific therapies include prostacyclins, phosphodiesterase inhibitors, endothelin receptor antagonists, and soluble guanylyl cyclase agonists [21]. Impaired synthesis of prostacyclin from arachidonic acid was the first pathobiological mechanism described in PAH, which pioneered effective drug development [12]. Reduced expression of prostacyclin synthase (PGIS) was found in pulmonary arteries in PAH patients [67], and peroxynitrite-mediated nitration of specific protein tyrosine residues was shown to be responsible for selective inactivation of PGIS and development of endothelial dysfunction [82,14].

Increased production of ROS and oxidative stress (OS) are the major contributors to the disorders in the L-arginine-NO-signaling pathway with changes in NO synthase (NOS) activity and NO deficiency and impaired pulmonary vasodilatory responses being characteristic features of PAH [23]. The underlying mechanisms of the derangements in NO synthesis are related either to the oxidative loss of NOS cofactor tetrahydrobiopterin (BH4) or oxidative attack on the zinc tetrathiolate cluster, which is an important mediator for the NOS dimeric structure [1]. Moreover, increased nonenzymatic NO production in hypoxic and oxidative environment rather promotes formation of peroxinitrite than improves NO bioavailability.

OS is commonly recognized player at the field of PAH. Signs of OS with excessive lipid peroxidation were demonstrated in PAH patients by increased levels of isoprostanes in urine [13] and malondialdehyde in plasma [32]. The other product of lipid peroxidation is hydroxynonenal (HNE). It is regarded not only as a useful marker of oxidative inflammatory condition but also exerts a wide range of pathophysiological effects, including synthesis and release of vasoactive mediators, breakdown of the endothelial barrier function and induction of the pro-inflammatory phenotype within the vessel wall making the study of HNE role in PAH particularly important [7]. The opposing effects of HNE on the NO homeostasis have been described [56], although the data on its role in PAH is missing.

Changes in the fatty acid (FA) composition were shown to interfere with NO signaling. NO production can be stimulated by the polyunsaturated FA (PUFA) such as arachidonic, eicosapentanoic (EPA), and docosahexanoic (DHA) acids [43,3,39]. This relationship is bidirectional as inhibition of NO synthesis was demonstrated to increase erythrocyte membrane fluidity due to the higher content of the unsaturated FAs [16], while administration of L-arginine increased levels of linoleic (LA), γ-linolenic acid (GLA) and dihomo-GLA [45]. Increased levels of free FAs in blood have been closely linked to endothelial dysfunction and altered eNOS activity attributed to the impaired action of the phospholipase C activating receptor agonists (such as acetylcholine, ATP, bradykinin) and reduced $[Ca^{2+}]_i$ increments [17,64]. Thus, metabolic alterations contributing to the impaired NO bioavailability observed in PAH involve alterations in the FAs status and potentiation of the L-arginine-NO-system during treatment may be accompanied by changes in the lipid composition.

At present, targeting L-arginine-NO pathway is a cornerstone strategy in PAH treatment. The approved therapeutic options include sildenafil, tadalafil, and vardenafil – inhibitors of phosphodiesterase type 5 (PDE-5), and riociguat, which stimulates soluble guanylyl cyclase and promotes cGMP synthesis [55,21]. Sildenafil has the highest level of evidence in PAH [21]. As the other PDE-5 inhibitors it blocks enzymatic degradation of the second messenger cGMP to GMP, increases intracellular cGMP level and leads to activation of the protein kinase G (PKG) with subsequent relaxation of smooth muscle cells, reduction of vascular remodeling and vasoconstriction. At the same time, precise molecular mechanisms of the protective effects of sildenafil are not completely understood. Recently, it was demonstrated that sildenafil suppressed multiple cytokines involved in neutrophil and mononuclear cell recruitment and reduced NF-kB and p38 MAPK activation in lungs from rats with monocrotaline induced PAH [36]. Moreover, its use caused stimulation of another PDE type 3-PKA pathway and targeted mitochondrial K^+_{ATP} channels, mitochondria and inflammation in hypertrophied right ventricle in PAH patients [48]. The inhibitory action of sildenafil on the main sources of free radicals production such as xanthine oxidase [63] and NADPH oxidase [47] points to its role as an antioxidant. Thus, the action of sildenafil is not limited to mechanistic inhibition of PDE5-PKG system but also includes modulation of the inflammatory and redox status, which are increasingly recognized as important contributors to PAH progression.

Development of PAH is characterized not only by the defective vasodilator but also exaggerated vasoconstrictor responses. The latter involve activation of the sympathetic nervous system, which was confirmed by the studies of the heart rate variability (HRV) in PAH subjects [6,71]. Interestingly, that inhibition of NOS in healthy volunteers caused sympathetic activation [76], while administration of PDE-5 inhibitor improved NO bioavailability and prevented hypoxic pulmonary hypertension [42]. There is increasing evidence that HRV is informative not only about the autonomic balance but also reflects the severity of the inflammatory conditions and depth of the oxidative stress [74] which has not been addressed in PAH.

In present study we aimed to assess the effects of sildenafil on the FAs status, level of hydroxynonenal (HNE) and heart rate variability (HRV) in PAH patients. We hypothesize that improvement of NO bioavailability with sildenafil will reduce lipid peroxidation, diminish signs of OS, providing antioxidant effect in PAH patients. Correction of the metabolic disorders at the level of an organism can be reflected by increased HRV and improvement in the balance between autonomic components which influence the heart rhythm.

2. Materials and methods

2.1. Selection of patients and study design

Patients with newly diagnosed PAH (idiopathic, familial or occurring after surgical repair of congenital systemic-to-pulmonary shunts that had been performed at least five years previously) with a six-minute walking distance from 100 m to 450 m were included into the study. PAH was characterized by the presence of pre-capillary PH, i.e., mPAP $\geq$ 25 mmHg, pulmonary artery wedge pressure $\leq$ 15 mmHg by right heart catheterization, in the absence of other causes of pre-capillary PH. Thermodilution method was used for cardiac output measurement. Upon confirmation of PAH sildenafil at a dose of 25 mg three times per day was initiated in addition to the conventional therapies including diuretics and anticoagulants. The duration of the study period was 12 weeks. Patients treated with other PAH specific medications such as inhaled or intravenous prostanoids, endothelin receptor blocker, calcium channel blockers and supplementation with L-arginine, and fish oil were not included into the study. Patients remained on their usual diet throughout the study. Healthy volunteers comprised a control group. They underwent standard clinical evaluation, blood sampling and HRV measurement once and did not receive any medications. The study was approved by Ethics Committee of the Danylo Halytsky Lviv National Medical University and written informed consent was obtained from all participants.