Research Results in Pharmacology

Research Results in Pharmacology 7(3): 23–31
UDC 616.131 008.331.1 07 08(072); 615.456.3; 611.08$=57.08
DOI 10.3897/rrpharmacology.7.67291

Research Article

Ubiquinol ameliorates endothelial dysfunction and increases expression of miRNA-34a in a rat model of pulmonary hypertension

Tatyana A. Kuropatkina[1], Nadezda V. Pankova[2], Natalia A. Medvedeva[3], Oleg S. Medvedev[1]

1 *Lomonosov Moscow State University, School of Medicine, Department of Pharmacology, Lomonosovsky prospect, Moscow 119991, Russia*

2 *Institute of Biomedical Problems, Russian Academy of Sciences, 76A Khoroshovskoe Shosse, Moscow 123007, Russia*

3 *Lomonosov Moscow State University, Faculty of Biology, Department of Human and Animal Physiology, 1-12 Leninskie gory, Moscow 119234, Russia*

Corresponding author: Tatyana A. Kuropatkina (0sylphide0@gmail.com)

Academic editor: Oleg Gudyrev ♦ **Received** 27 April 2021 ♦ **Accepted** 15 July 2021 ♦ **Published** 13 September 2021

Citation: Kuropatkina TA, Pankova NV, Medvedeva NA, Medvedev OS (2021) Ubiquinol ameliorates endothelial dysfunction and increases expression of miRNA-34a in a rat model of pulmonary hypertension. Research Results in Pharmacology 7(3): 23–31. https://doi.org/10.3897/rrpharmacology.7.67291

Abstract

Introduction: In this research, we evaluate the effect of intravenously administrated solubilized ubiquinol on 4-week monocrotalin-induced pulmonary hypertension (PH) in rats.

Materials and methods: To reproduce the model, some male Wistar rats were subcutaneously injected with alcohol solution of monocrotaline 60 mg/kg and the rest – with alcohol solution (Control). Those with monocrotaline (MCT) were divided into 3 groups. They underwent intravenous administration of 1% ubiquinol solution 30 mg/kg (MCT-Ubiquinol), the vehicle (MCT-Vehicle) and saline (MCT-saline) three times on days 7, 14 and 21, depending on the group. The hemodynamic parameters were measured in anesthetized rats on day 29. Right ventricle hypertrophy, pulmonary arteries reactivity and expression of miRNA-21 and miRNA-34a were estimated after euthanasia.

Results and discussion: All MCT-groups demonstrated an increase in right ventricle systolic pressure and hypertrophy in comparison with the control group. An increase in lung weight was shown in MCT-Vehicle and MCT-Saline; however, the MCT-Ubiquinol indicators did not differ from those of the Control. There was an increased vasodilatation response to acetylcholine at concentrations of $1*10^{-6}$M and $1*10^{-5}$M in MCT-Ubiquinol in contrast to the other two MCT-groups. A significantly lower level of expression of miRNA-34a was observed in MCT-Ubiquinol.

Conclusion: Our findings suggest that a triple ubiquinol injection influences pulmonary changes and endothelium-depended vasodilatation, which contributes to pulmonary vascular tone and reactivity. A decrease in miRNA-34a expression in MCT-Ubiquinol group demonstrates the ubiquinol anti-inflammatory properties.

Keywords

MCT-induced pulmonary hypertension, CoQ_{10}, ubiquinol, oxidative stress, inflammation, endothelial dysfunction, miRNA-34a.

Introduction

Coenzyme Q_{10} (CoQ_{10}) is an endogenous component of the mitochondrial respiratory chain of almost any cell in the body. CoQ_{10} can be regarded as an antioxidant due to its redox potential. Antioxidant properties of CoQ_{10} are mediated mainly by neutralization of reactive oxygen species (ROS) by ubiquinol and prevention of oxidative stress development. Oxidative stress is closely related to inflammation, and one can be easily caused by the other. Thus, the biological role of ubiquinol may also be determined by its possible anti-inflammatory activity (Bessler et al. 2010; Fan et al. 2017; Liu et al. 2017).

Many studies have shown the therapeutic efficacy of CoQ_{10} for chronic heart failure (Langsjoen and Langsjoen 2008; Littarru et al. 2011; Sharma et al. 2016), coronary heart disease (Kuimov and Murzina 2013; Ayers et al. 2018), and a positive effect of CoQ_{10} on the prognosis of pulmonary hypertension (Sharp et al. 2014) and endothelial vascular dysfunction (Littarru et al. 2011; Gao et al. 2012; Sharma et al. 2016). However, it should be noted that oral CoQ_{10} administration is characterized by rather low bioavailability at the level of only 0.1–3% (Kharitonova et al. 2013). In this connection, soluble form of ubiquinol for intravenous administration was developed to overcome the problem of its low bioavailability (Kharitonova et al. 2013; Kalenikova et al. 2017; Shapoval et al. 2018). The intravenous form provides an instant increase in plasma level of CoQ_{10} and gradual replenishment of its tissue level. This fact is demonstrated in a rapid and long-lasting correction of the redox imbalance in oxidative stress and accompanying pathologies (Kalenikova et al. 2015; Shapoval et al. 2018).

Pulmonary hypertension (PH) is defined as a chronic disease with remodeling of small and medium pulmonary vessels, which leads to an increase in pulmonary blood pressure, heart failure and, ultimately, possible death (Rubin 1997). Morphological changes are mainly inflammatory in character in the chronic form of PH and include apoptosis-resistant proliferation of endothelium and smooth muscle cells of the pulmonary artery. Inflammation reduces the activity of NO-synthase in endothelial cells, increases the permeability of the endothelium, and increases its sensitivity to vasoconstrictors (Schermuly et al. 2011; Thenappan et al. 2018). Eventually, it leads to the narrowing of arterioles lumen and compensatory hypertrophy of the right ventricle (RV) with subsequent disruption of its function. In addition to proliferative changes in vessels walls due to inflammation accompanied with oxidative damage, the endothelial dysfunction develops (Schulz et al. 2011), which also contributes significantly to the increasing pressure in the pulmonary artery. Thus, oxidative stress and inflammation in endothelial cells and endothelial dysfunction are among the main factors in the pathogenesis of PH.

Nowadays there is a large number of studies showing the critical role of miRNAs as markers of cell proliferation (Wang et al. 2013), inflammation processes (Sessa 2011), apoptosis (Fu et al. 2018), and other reactions. A number of miRNAs mediating the proliferation and migration of smooth muscle cells (miR-21, miR-124, miR-17–92, etc.) in PH have been identified, some of which may also participate in the proliferation of endothelial cells and fibroblasts (Boucherat et al. 2015; Zhou et al. 2015). A group of miRNAs involved in the development of the inflammatory process accompanying PH (miR-124, miR-223, miR-34a, miR-132, etc.) has also been identified, but they are poorly studied (Zhou et al. 2015). Thus, the expression of sensitive miRNAs can be considered as a potential therapeutic target for research of various drugs, including CoQ_{10}.

A significant obstacle faced when studying the CoQ_{10} effect on cardiovascular diseases (CVD) is the way it is administered. In most studies, the drug was administered orally (Sharp et al. 2014; Fan et al. 2017; Ayers et al. 2018), which leaves open the question of how effective CoQ_{10} is when its bioavailability is gradually increased. Therefore, in this study we used water soluble form of ubiquinol for intravenous administration.

As noted above, CoQ_{10} administration has a therapeutic effect in various cardiovascular diseases (CVDs), including PH. Therefore, in this study we have tested the hypothesis that the reduced form of CoQ_{10} (ubiquinol) can reduce a degree of PH progression, which is based on the antioxidant properties of ubiquinol. It was shown that the oxidative vascular damage can disrupt the function of NO-synthase (Schulz et al. 2011; Silva et al. 2012). As the result, the level of NO decreases, leading to an increase in vascular resistance. Hence, ubiquinol as an antioxidant can potentially affect these triggers of PH development by lowering the level of ROS. The aim of this work was to evaluate the effect of intravenous administration of new solubilized form of ubiquinol on the development of endothelial dysfunction and on the biomarkers of inflammation.

Materials and methods

Animals and experimental design

The experiments were carried out on the 2-month-old male Wistar rats (weight 180–230 g). All the manipulations with the animals were carried out according to the Council Directive 86/609/EEC principles. The animals were obtained from the vivarium of the Research Institute of General Pathology and Pathophysiology (Moscow, Russia). The rats were kept under the 12-hour daylight conditions with the free access to water and food.

The experiment involved 4 groups, with 15 animals in each group. On the first day of the experiment, the animals were injected with a single subcutaneous injection of MCT (60 mg/kg) in three groups. In the fourth group, the animals were injected with a monocrotaline solvent (alcohol solution), i.e. the control group to MCT injection was regarded as a group without PH (Control).

MCT-induced PH model was chosen as the most convenient, technically simple and reproducible. It includes

all special links: vascular remodeling, smooth muscle cell proliferation of pulmonary vessels and changes in the level of pro-inflammatory markers (Gomez-Arroyo et al. 2012).

On days 7, 14 and 21 after MCT administration, the animals were divided into 3 groups. The group MCT-Ubiquinol was injected into the tail vein with 1% solubilized ubiquinol at a dose of 30 mg/kg; MCT-Vehicle was administered only a vehicle without ubiquinol 0.9 ml/kg (the vehicle includes solubilizer and 0.05–0.2% of direct antioxidants that may also have an impact, i.e. it was the control to the use of ubiquinol), and MCT-Saline was injected with 0.9% NaCl solution 0.9 ml/kg (it was the control to the healthy rats showing the influence of MCT and at the same time to MCT-Vehicle). The control group of healthy rats was also injected with a vehicle. Water soluble ubiquinol and vehicle were provided by Scientific Production Association "House of Pharmacy", St. Petersburg, Russia (patent #RU2635993-C1). Thus, a total of 4 groups were formed: one normotensive (Control) and 3 hypertensive groups (MCT-Ubiquinol, MCT-Vehicle and MCT-Saline).

It is important to note that our previous study did not reveal any effects of intravenous injection of ubiquinol or its vehicle on any of the PH diagnostic parameters in healthy rats, which is why no data from it are included here.

Measurement of hemodynamic parameters

Hemodynamic parameters were measured on the 29th day after injection of MCT. The animals were anesthetized with urethane (water solution, 1.2 g/kg, 0.6 g/ml) intraperitoneally. Mean systemic arterial pressure and right ventricle systolic pressure (RVSP) were estimated directly by a Statham transducer (Statham Instrument Inc., USA), an operational amplifier and a multichannel analog-to-digital converter L-Card E14-140 (Russia). For this purpose, a PE10 catheter was inserted into the femoral artery and a PE 50 catheter (Medsil, Russia) – through the right jugular vein of the anesthetized rats was placed in the right ventricle (RVSP). The level of RVSP was used to evaluate severity of pulmonary hypertension.

Morphometric measurement

A morphological study of myocardium was carried out after registration of the hemodynamic indices. After euthanasia, the heart was taken out and washed in a saline solution; the atrium was cut out, and the left ventricle was separated from the right ventricle and an interventricular septum. An assessment of a degree of right ventricular (RV) hypertrophy was carried out relative to the sum of left ventricular (LV) and interventricular septum (S) weights (RV/(LV+S)). The lung weight was also measured.

Measurement of the pulmonary arteries reactivity

Right after euthanizing by decapitation, a segment of a similar size was isolated from the 3rd order branches of the pulmonary artery of each animal. In order not to damage the vessel, the segment was isolated and fixed on a perfusion needle (with internal diameter of 0.5 mm) together with the surrounding tissues. The isolated vessels were perfused with a modified Krebs-Hensleit physiological solution (content of substances in mM: NaCl – 118, KCl – 4.7, $CaCl_2$ – 3.3, $MgSO_4$ – 2.4, KH_2PO_4 – 1.18, glucose – 5.05, and $NaHCO_3$ – 24.9; pH 7.4). Perfusion was performed at a constant internal flow rate of 2 ml/min and external flow rate of 4 ml/min. The reaction of vessels was evaluated by the changes in perfusion pressure measured by a Statham transducer (Statham Instrument Inc., USA), which was recorded by means of PowerGraph 3.0 software (Russia). Temperature of perfusion solution was maintained at 37–37.5 °C. The recording of perfusion pressure was started 20 minutes after stabilization of vascular tone. Serotonin was used to create a contractile tone as a vasoconstriction agent at a concentration of $3*10^{-6}$M. Dose-dependent vasodilatation was measured on acetylcholine (ACh) in a concentration ranging from $1*10^{-9}$M to $1*10^{-5}$M. The solution was changed to a more concentrated one after a response having reached the plateau.

miRNA measurement

After decapitation, a heart was cut out; a RV was separated from the LV and the interventricular septum, and then weighed. After being isolated and weighed, the right ventricle tissue was immediately frozen in liquid nitrogen and stored at -80 °C. Total RNA extraction was carried out after tissue homogenization, using a set of RNA extraction kits (Sintol, Russia) according to the manufacturer's instructions.

The levels of expression of miRNA were determined in two stages. During the first stage, the reverse transcription of RNA was performed, using a miScript II RT Kit (Qiagen GmbH, Germany) in accordance with the manufacturer's instructions. The second stage of real-time quantitative Polymerase Chain Reaction (PCR) was performed using a SYBR Green PCR Kit (Qiagen GmbH, Germany). PCR cycles were performed on a Real-Time CFX96 Touch amplifier (Bio-Rad Laboratories, Inc., USA). PCR was performed under the following conditions: primary denaturation – 95 °C/5 minutes; then 50 cycles, each included denaturation – 94 °C/15 seconds, annealing – 55 °C/30 seconds and elongation (extension) of 70 °C/30 seconds. All the reactions were made in three recurrences.

The expression levels of miRNA-21 and miRNA-34a were calculated by the ΔΔCt method. Small nuclear RNAs U6 (U6 small nuclear RNAs) were used as internal control. As a result of processing the measurements, the values of gene expression levels were obtained in the samples of experimental groups relative to miRNA in the control group.

Bioethical approvement

The research protocol was approved by Bioethics commission of Moscow State University, Institute of Biology (Protocol №113-G, 19.06.2020)

Data analysis

A statistical analysis was performed by Student's t-test, one-way and two-way ANOVA, using GraphPad Prism 8 software. All the data are represented as the mean value ± standard error of the mean (SEM) of n experiments. EC_{50} – the logarithm of the acetylcholine concentration producing 50% of the E_{max} – was calculated by non-linear regression analysis, with E_{max} being a maximum relaxation response to acetylcholine ($1*10^{-5}$). Correlation was analyzed using Spearman correlation coefficient.

The differences were considered statistically significant at the permissible error probability of $p \leq 0.05$.

Results and discussion

Systemic blood pressure and heart rate measurement did not reveal any statistically significant differences between the animals of all the groups (Table 1).

Table 1. Hemodynamic Parameters and Morphometry in All Experimental Groups by the End of 4-week MCT-induced PH (Systemic Blood Pressure, Heart Rate, Right Ventricular Systolic Pressure and Relative Weight of the Right Ventricle of the Heart in Rats of All Experimental Groups)

Group	Parameter			
	Systemic blood pressure, mm Hg	Heart rate, beats/min	Right ventricular systolic pressure, mm Hg	RV/(LV+S) ratios
Control (n = 12)	98.3 ± 3.24	354.2 ± 10.31	38.6 ± 1.45	0.30 ± 0.03
MCT-Ubiquinol (n = 11)	100.2 ± 5.0	342.4 ± 17.40	58.27 ± 2.96*	0.46 ± 0.03$^{\$}$
MCT-Vehicle (n = 10)	97.4 ± 2.6	362.9 ± 11.65	57.58 ± 2.89$^{\#}$	0.44 ± 0.03$^{\epsilon}$
MCT-Saline (n = 8)	95.8 ± 4.7	359.1 ± 13.4	58.38 ± 3.45$^{\&}$	0.46 ± 0.02^{p}

Note: $^{*,\#,\&,\$,\epsilon,p}$ MCT-groups vs Control, $p \leq 0.01$, one-way ANOVA; Abbreviations: RV – right ventricle mass, LV – left ventricle mass, S – interventricular septum.

In the animals injected with MCT, these RVSP values were statistically significantly higher – on average by 1.5 times – compared to the control group ($p \leq 0.05$, Table 1), which indicates the development of PH in these groups. However, the RVSP values did not statistically differ between the experimental groups.

The measurement of a RV hypertrophy degree demonstrated similar changes: in the MCT groups, the RV was significantly higher in comparison with that of the Control group ($p \leq 0.01$, Table 1). It should be noted that the direct correlation between the increased RVSP parameters and the degree of RV hypertrophy was observed only in the group of MCT-Saline ($r = 0.9524$, $p \leq 0.05$).

Lung weight of rats in the MCT-Vehicle and MCT-Saline groups was higher in comparison with that in the Control ($p \leq 0.05$, Figure 1). For the MCT-Ubiquinol group, no differences of this kind were observed.

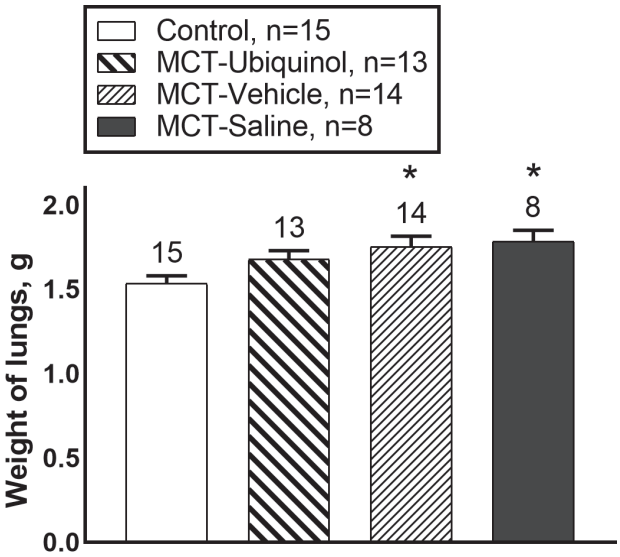

Figure 1. Lung mass in all experimental groups by the end of 4-week MCT-induced PH. Note: *MCT-Vehicle, MCT-Saline vs Control, $p \leq 0.05$, one-way ANOVA.

The vasodilatation effect of pulmonary arteries to acetylcholine ($1*10^{-9}$-$1*10^{-5}$M) in the groups with MCT-induced PH was significantly lower in comparison with that in the Control group. In the MCT-Ubiquinol, MCT-Vehicle and MCT-Saline groups, the difference was found in the concentrations ranging from $1*10^{-7}$ to $1*10^{-5}$M. The ubiquinol injection resulted in a significant increase in the vasodilatation response to acetylcholine at a concentration of $1*10^{-6}$M (-14.05 mm Hg) by 13.5% compared to the MCT-Vehicle group (-10.76 mm Hg) and by 14.8% compared to MCT-Saline (-12.17 mm Hg); at $1*10^{-5}$M (-16.13 mm Hg) by 14.3% (-12.82 mm Hg) and 17.4% (-13.44 mm Hg), respectively ($p \leq 0.05$). At $1*10^{-7}$M, there were significant differences in the experimental groups only with the Control group (Fig. 2A).

E_{max} values in the experimental groups significantly differ from those in the Control. However, no significant changes in vascular sensitivity (EC_{50}) to acetylcholine were found (Fig. 2B, Table 2).

The expression levels of miRNA-21 and miRNA-34 in

Table 2. EC_{50} and E_{max} of Rat Pulmonary Arteries to Acetylcholine ($1*10^{-9}$-$1*10^{-5}$M)

Parameter	Group			
	Control	MCT-Ubiquinol	MCT-Vehicle	MCT-Saline
Emax, %	39.20 ± 0.73*	33.95 ± 0.61$^{\#}$	26.06 ± 1.41	26.88 ± 1.16
EC_{50}	$4.25 * 10^{-8}$	$7.22 * 10^{-8}$	$5.38 * 10^{-8}$	$3.47 * 10^{-8}$

Note: *Control vs MCT-Ubiquinol, MCT-Vehicle, MCT-Saline, $p \leq 0.05$; $^{\#}$MCT-Ubiquinol vs MCT-Vehicle, MCT-Saline, $p \leq 0.05$, one-way ANOVA.

RV tissue samples significantly differ in the experimental groups in comparison with those in the Control ($p \leq 0.05$). The expression of miRNA-21 in MCT-Ubiquinol increased by 3.92 times, in MCT-Vehicle – by 4.26 times, and in MCT-Saline – by 4.29 times. However, the groups did not differ significantly between each other.

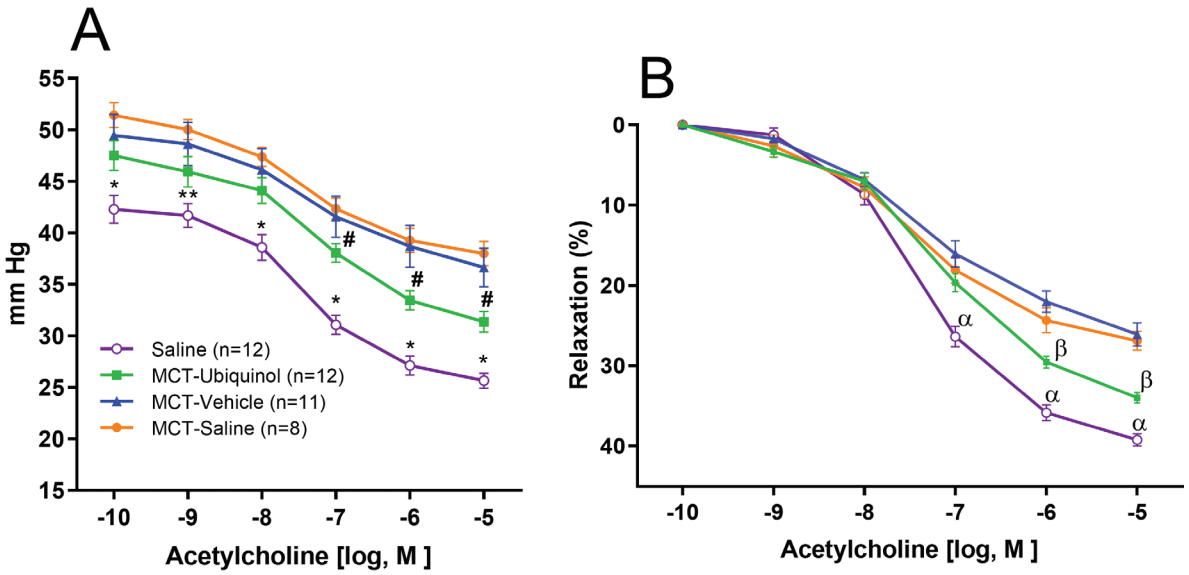

Figure 2. Dose-dependent decrease in the perfusion pressure in the isolated pulmonary artery response to perfusion with acetylcholine in rats of all experimental groups by the end of 4-week MCT-induced PH; A – response in mm Hg; B – response in %. **Note**: *,αControl vs MCT-groups, $p \leq 0.05$; **Control vs MCT-Vehicle, MCT-Saline, $p \leq 0.05$; #,βMCT-Ubiquinol vs MCT-Vehicle MCT-Saline, $p \leq 0.05$, two-way ANOVA.

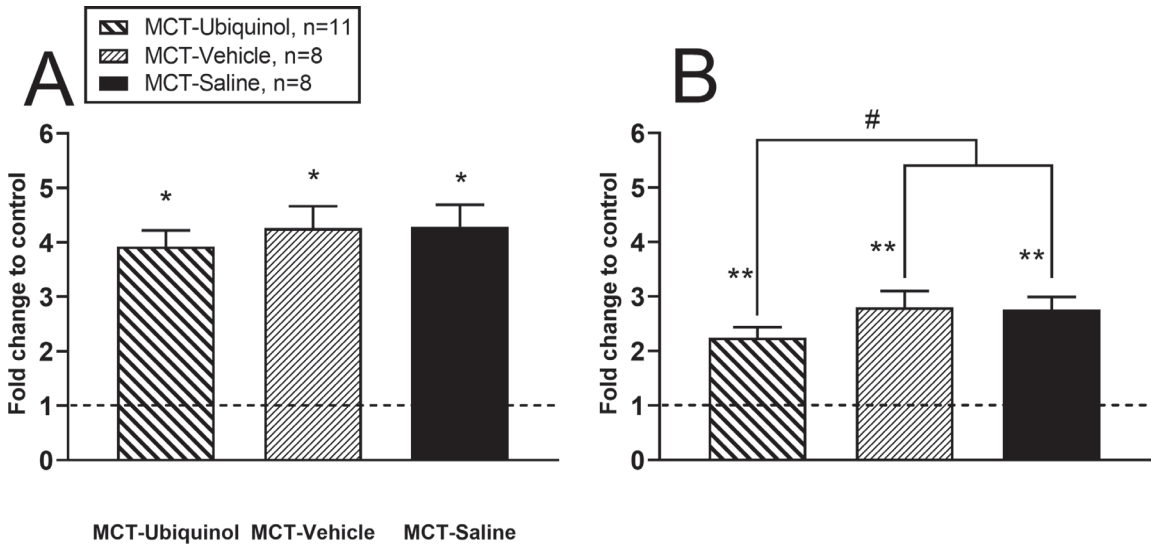

Figure 3. Relative miR-21 (A) and miR-34a (B) expression levels in RV tissue of rats with 4-week MCT-induced PH. **Note:** *, **MCT-groups vs Control, $p \leq 0.05$; #MCT-Ubiquinol vs MCT-Vehicle, MCT-Saline, $p \leq 0.05$; --- reference line of Control group, n = 9, Mann-Whitney test.

The expression of miRNA-34a was significantly higher in all the experimental groups in comparison with the Control. However, the miRNA-34a expression was significantly lower in the animals with ubiquinol injection: miRNA-34a expression was 2.24 times higher for MCT-Ubiquinol, while for MCT-Vehicle and MCT-Saline it was 2.80 and 2.76 times higher, respectively, compare to the rate of the Control.

The expression level of miRNA-21 directly correlated with the increase of RV mass ($p \leq 0.05$). For miRNA-34a such a correlation was not found.

Discussion

One of the characteristics of pulmonary hypertension is the endothelial dysfunction in the small circle (Xu and Erzurum 2011; Thenappan et al. 2018). It leads to an increase in pulmonary vascular resistance and wall enlargement as well as an increase in the vascular basal tone (Nemery et al. 1983). During the study of pulmonary vascular reactivity, significantly higher values of the initial tone in the animals injected with MCT were

found. The three-time injection of water soluble ubiquinol in the animals with 4-week MCT-induced PH contributed to the correction of this pathological state and an increase in endothelium-dependent dilatation to acetylcholine. Similar results were obtained earlier on a less severe model of 3-week MCT-induced PH, where the protective effect of ubiquinol on RV hypertrophy had also been demonstrated (Abdullaev et al. 2019). These results indicate that administration of ubiquinol helps to restore the pathological reactivity of pulmonary arteries to vasoactive factors, even in case of severe PH.

The balance between the levels of vasoconstrictors and vasodilators originates from increased permeability of the smooth muscle layer and damage to the endothelium. (Christman et al. 1992; Wharton et al. 2005; Ghofrani et al. 2006). The production of NO, one of the most important vasodilating factors, in endothelium is controlled by endothelial NO synthase (eNOS). Under physiological conditions, even a small amount of NO can have a powerful vasodilator effect, but in oxidative stress conditions, much higher levels of NO excretion may have a negligible effect on vascular tone and even contribute to oxidative damage to endothelium. The interaction of ROS with NO disrupts eNOS, causing peroxynitrite synthesis, and lowers the bioavailability of already released NO, which aggravates endothelial dysfunction (Münzel et al. 2005). Thus, the bioactivity of NO is a more important parameter than just a level of eNOS expression or NO release (Vásquez-Vivar et al. 1998).

One of the possible mechanisms of CoQ_{10} action in CVDs is its ability to improve endothelial function and facilitate vascular dilation (Kumar et al. 2009; Huo et al. 2018). Being in the inner mitochondria membrane, CoQ_{10} increases NO production and its bioavailability (Sabbatinelli et al. 2020), and reduces its oxidation to peroxinitrites. It has also been shown that CoQ_{10} is capable of modulating the activity of both endothelial and inducible NO synthase (iNOS), providing an optimal non-toxic level of NO (Tsai et al. 2012).

It is known that the PH pathogenesis also involves an increase in pro-inflammatory cytokines IL-1β, IL-6 (Humbert et al. 1995; Steiner et al. 2009; Groth et al. 2014) and TNF-α (Steiner et al. 2009; Groth et al. 2014). Oxidative stress that accompanies PH and ROS formation activates NF-κB, which in turn increases the expression of TNF-α and IL-6 (Schmelzer et al. 2008; Wang et al. 2013). It is also worth noting that the role of NO is, on the one hand, prevention of activation of NF-κB and subsequent production of inflammatory mediators, which promote leukocyte adhesion (Chen et al. 2003), and activation of macrophages (Chen et al. 2004) on the other hand, ROS reduces the bioactivity of NO, which also contributes to the development of inflammation in the vascular wall and endothelial dysfunction. In this context, the biological significance of CoQ_{10} may be determined by its possible anti-inflammatory activity (Schmelzer et al. 2007; Schmelzer et al. 2009; Wang et al. 2013). The meta-analyses confirm that the CoQ_{10} administration decreases the level of pro-inflammatory cytokines (IL-6, TNF-α, C-reactive protein) (Gao et al. 2012; Zhai et al. 2017). A possible anti-inflammatory effect of CoQ_{10} is explained by inhibition of NF-κβ expression (Schmelzer et al. 2007; Wang et al. 2013), which in turn is mediated by the antioxidant effect of CoQ_{10} on ROSs which further activate the expression of NF-κβ.

Oxidative damage also affects the structure of lung tissue: growing fibrosis prevents adequate ventilation and perfusion of the lungs, causing even greater vascular constriction and hypoxia. Fibrosis occurs not only due to increased synthesis of pro-inflammatory cytokines (Kumar et al. 2009; Groth et al. 2014; Huo et al. 2018), but also due to one of the leading factors of fibrogenesis – connective tissue growth factor (CTGF). In our work, both on a mild 3-week model and a 4-week model (Abdullaev et al. 2019), no reliable differences in lung weight were observed between the MCT-Ubiquinol group and the Control. This may indicate relief of PH symptoms in rats of this group and may be associated with suppression of CTGF due to the use of CoQ_{10}, which is confirmed by other studies (De Blasio et al. 2015).

A certain data on miRNA as biomarkers of PH and possible influence of various agents on their expression (Zhou et al. 2015; Gubrij et al. 2016; Batkai et al. 2017) created our interest to investigate miRNA as a potential target for the effects of CoQ_{10}. Since the pathogenesis of MCT-induced pulmonary hypertension has significant inflammatory and proliferative components (Boucherat et al. 2015; Zhou et al. 2015), miRNA-21 was selected as a marker of RV hypertrophy, and miRNA-34a – as an indicator of inflammation in RV tissue.

In our study, despite the fact that the use of ubiquinol did not influence the expression of miRNA-21, an increase in its level in all the MCT-groups corresponds the development of RV hypertrophy and may be regarded as a possible therapeutic target.

The evaluation of the expression level of the miRNA-34 inflammatory marker showed that ubiquinol may have a positive effect on the level of PH development. Earlier, Schmelzer C. et al. (2007, 2009) reported that ubiquinol may reduce the expression of pro-inflammatory markers, proving that the effect of the drug occurs on the level of modulation of NF-κβ expression (Pileczki et al. 2016).

Conclusion

A decrease in the expression of miRNA-34 in the animals injected by ubiquinol suggests that the recovery of the reactivity of pulmonary vessels is associated with a

decrease in inflammation in these animals. An indirect argument in favor of this assumption is a lower degree of an increase in the lung weight in these animals in comparison with other groups with MCT-induced PH, the lung weight of which increased significantly.

A possible explanation for the positive effect of intravenous injection of ubiquinol on MCT-induced PH progression in rats could be the restoration of ubiquinol pool in the body, which is confirmed by the results of a study conducted by our colleagues earlier (Kalenikova et al. 2016).

Conflict of interest

The authors declare no conflict of interests.

Acknowledgement

This research received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

References

- Abdullaev Sh, Kuropatkina T, Pan'kova N, Artem'eva M, Makarov V, Medvedeva N, Medvedev O (2019) Intravenous administration of ubiquinol influences the development of monocrotaline pulmonary hypertension in rats. Experimental and Clinical Pharmacology [Eksperimentalnaya i Klinicheskaya Pharmakologiya] 82(5): 20–23. https://doi.org/10.30906/0869-2092-2019-82-5-20-23 [in Russian]
- Ayers J, Cook J, Koenig R, Sisson E, Dixon D (2018) Recent developments in the role of coenzyme Q_{10} for coronary heart disease: a Systematic review. Current Atherosclerosis Reports 20(6): 29. https://doi.org/10.1007/s11883-018-0730-1 [PubMed]
- Batkai S, Bär C, Thum T (2017) MicroRNAs in right ventricular remodelling. Cardiovascular Research 113(12): 1433–1440. https://doi.org/10.1093/cvr/cvx153 [PubMed]
- Bessler H, Bergman M, Blumberger N, Djaldetti M, Salman H (2010) Coenzyme Q10 decreases TNF-alpha and IL-2 secretion by human peripheral blood mononuclear cells. Journal of Nutritional Science and Vitaminology 56(1): 77–81. https://doi.org/10.3177/jnsv.56.77 [PubMed]
- Boucherat O, Potus F, Bonnet S (2015) MicroRNA and pulmonary hypertension. Advances Experimental Medicine Biology 888: 237–252. https://doi.org/10.1007/978-3-319-22671-2_12 [PubMed]
- Chen X, Zhang Q, Zhao R, Ding X, Tummala P, Medford R (2003) Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. Journal of Pharmacology and Experimental Therapeutics 305(2): 573–580. https://doi.org/10.1124/jpet.102.047894 [PubMed]
- Chen X, Zhang Q, Zhao R, Medford R (2004) Superoxide, H_2O_2, and iron are required for TNF-alpha-induced MCP-1 gene expression in endothelial cells: role of Rac1 and NADPH oxidase. American Journal of Physiology Heart and Circulatory Physiology 286(3): H1001–H1007. https://doi.org/10.1152/ajpheart.00716.2003 [PubMed]
- Christman B, McPherson C, Newman J, King G, Bernard G, Groves B, Loyd J (1992) An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. New English Journal of Medicine 327: 70–75. https://doi.org/10.1056/NEJM199207093270202 [PubMed]
- De Blasio MJ, Huynh K, Qin C, Rosli S, Kiriazis H, Ayer A, Cemerlang N, Stocker R, Du XJ, McMullen JR, Ritchie RH (2015) Therapeutic targeting of oxidative stress with coenzyme Q10 counteracts exaggerated diabetic cardiomyopathy in a mouse model of diabetes with diminished PI3K(p110α) signaling. Free Radical Biology and Medicine 87: 137–147. https://doi.org/10.1016/j.freeradbiomed.2015.04.028 [PubMed]
- Fan L, Feng Y, Chen GC, Qin LQ, Fu CL, Chen LH (2017) Effects of coenzyme Q10 supplementation on inflammatory markers: A systematic review and meta-analysis of randomized controlled trials. Pharmacological Research 119: 128–136. https://doi.org/10.1016/j.phrs.2017.01.032 [PubMed]
- Fu J, Chen Y, Li F (2018) Attenuation of MicroRNA-495 derepressed PTEN to effectively protect rat cardiomyocytes from hypertrophy. Cardiology 139(4): 245–254. https://doi.org/10.1159/000487044 [PubMed]
- Gao L, Mao Q, Cao J, Wang Y, Zhou X, Fan L (2012) Effects of coenzyme Q10 on vascular endothelial function in humans: a meta-analysis of randomized controlled trials. Atherosclerosis 221(2): 311–316. https://doi.org/10.1016/j.atherosclerosis.2011.10.027 [PubMed] [PMC]
- Ghofrani H, Osterloh I, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nature Review Drug Discovery 5(8): 689–702. https://doi.org/10.1038/nrd2030 [PubMed]
- Gomez-Arroyo J, Farkas L, Alhussaini A, Farkas D, Kraskauskas D, Voelkel N, Bogaard H (2012) The monocrotaline model of pulmonary hypertension in perspective. American Journal of Physiology-Lung Cellular and Molecular Physiology 302(4): L363–L369. https://doi.org/10.1152/ajplung.00212.2011 [PubMed]
- Groth A, Vrugt B, Brock M, Speich R, Ulrich S, Huber L (2014) Inflammatory cytokines in pulmonary hypertension. Respiratory Research 15(1): 47. https://doi.org/10.1186/1465-9921-15-47 [PubMed] [PMC]
- Gubrij I, Pangle A, Pang L, Johnson L (2016) Reversal of microRNA dysregulation in an animal model of pulmonary hypertension. PLoS ONE 11(1): e0147827. https://doi.org/10.1371/journal.pone.0147827 [PubMed]
- Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanaud P, Simonneau G, Emilie D (1995) Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine 151(5): 1628–1631. https://doi.org/10.1164/ajrccm.151.5.7735624 [PubMed]
- Huo J, Xu Z, Hosoe K, Kubo H, Miyahara H, Dai J, Mori M, Sawashita J, Higuchi K (2018) Coenzyme Q10 prevents senescence and dysfunction caused by oxidative stress in vascular endothelial cells. Oxidative Medicine and Cellular Longevity 2018: 3181759. https://doi.org/10.1155/2018/3181759 [PubMed]

- Kalenikova E, Gorodetskaya E, Kulyak O, Kozaeva L, Makarov V, Pozharitskaya O, Shikov A, Medvedev O (2017) Preclinical study of pharmacokinetics of a new medicinal form of ubiquinol for intravenous administration. Chemical-Pharmaceutical Journal [Khimiko-Farmatsevticheskii Zhurnal] 11(51): 3–7. https://doi.org/10.30906/0023-1134-2017-51-11-3-7 [in Russian]
- Kalenikova E, Gorodetskaya E, Tokareva O, Belousova M, Kulyak O, Artem'eva M, Medvedev O (2016) Multi-day monitoring of ubidecarenone level in rat plasma and tissues after a single intravenous injection. Pharmaceutical Chemistry Journal 49: 719. https://doi.org/10.1007/s11094-016-1360-y
- Kalenikova E, Kharitonova E, Gorodetskaya E, Tokareva O, Medvedev O (2015) HPLC estimation of coenzyme Q(10) redox status in plasma after intravenous coenzyme Q(10) administration. Biomedical Chemistry [Biomeditsinskaya Khimiya] 61(1): 125–131. https://doi.org/10.18097/PBMC20156101125 [PubMed] [in Russian]
- Kharitonova E, Kalenikova E, Gorodetskaya E, Medvedev O (2013) Pharmacokinetics of solubilized CoQ10 in composition of qudesan at parenteral injection. Siberian Medical Review [Sibirskii Meditsinskii Zhurnal] 6(84): 26–29. [in Russian]
- Kuimov A, Murzina T (2013) Coenzyme q10 in complex therapy of patients with ischemic heart disease. Cardiology [Kardiologiia] 53(8): 40–43. [PubMed] [in Russian]
- Kumar A, Kaur H, Devi P, Mohan V (2009) Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. Pharmacology & Therapeutics 124(3): 259–268. https://doi.org/10.1016/j.pharmthera.2009.07.003 [PubMed]
- Langsjoen P, Langsjoen A (2008) Supplemental ubiquinol in patients with advanced congestive heart failure. Biofactors 32: 119–128. https://doi.org/10.1002/biof.5520320114 [PubMed]
- Littarru G, Tiano L, Belardinelli R, Watts G (2011) Coenzyme Q(10), endothelial function, and cardiovascular disease. Biofactors 37(5): 366–373. https://doi.org/10.1002/biof.154 [PubMed]
- Liu H, Cheng S, Huang Y, Huang Y, Lin P (2017) Coenzyme Q10 and oxidative stress: Inflammation status in hepatocellular carcinoma patients after surgery. Nutrients 9(1): 29. https://doi.org/10.3390/nu9010029 [PubMed] [PMC]
- Münzel T, Daiber A, Ullrich V, Mülsch A (2005) Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. Arteriosclerosis, Thrombosis and Vascular Biology 25(8): 1551–1557. https://doi.org/10.1161/01.ATV.0000168896.64927.bb [PubMed]
- Nemery B, Wijns W, Piret L, Cauwe F, Brasseur L, Frans A (1983) Pulmonary vascular tone is a determinant of basal lung perfusion in normal seated subjects. Journal of Applied Physiology 54(1): 262–266. https://doi.org/10.1152/jappl.1983.54.1.262 [PubMed]
- Pileczki V, Cojocneanu-Petric R, Maralani M, Neagoe I, Sandulescu R (2016) MicroRNAs as regulators of apoptosis mechanisms in cancer. Clujul Medical 89(1): 50–55. https://doi.org/10.15386/cjmed-512 [PubMed] [PMC]
- Rubin L (1997) Primary pulmonary hypertension. New English Journal of Medicine 336: 111–117. https://doi.org/10.1056/NEJM199701093360207 [PubMed]
- Sabbatinelli J, Orlando P, Galeazzi R, Silvestri S, Cirilli I, Marcheggiani F, Tiano L (2020) Ubiquinol Ameliorates Endothelial dysfunction in subjects with mild-to-moderate dyslipidemia: A randomized clinical trial. Nutrients 12(4): 1098. https://doi.org/10.3390/nu12041098 [PubMed] [PMC]
- Schermuly R, Ghofrani H, Wilkins M, Grimminger F (2011) Mechanisms of disease: pulmonary arterial hypertension. Nature Reviews Cardiology 8(8): 443–455. https://doi.org/10.1038/nrcardio.2011.87 [PubMed]
- Schmelzer C, Lindner I, Rimbach G, Niklowitz P, Menke T, Döring F (2008) Functions of coenzyme Q10 in inflammation and gene expression. Biofactors 32(1–4): 179–183. https://doi.org/10.1002/biof.5520320121 [PubMed]
- Schmelzer C, Lorenz G, Rimbach G, Döring F (2007) Effects of coenzyme Q10 on TNF-α secretion in human and murine monocytic cell lines. Biofactors 31(3–4): 211–217. https://doi.org/10.1002/biof.5520310104 [PubMed]
- Schmelzer C, Lorenz G, Rimbach G, Döring F (2009) In vitro effects of the reduced form of coenzyme Q(10) on Secretion levels of TNF-alpha and chemokines in response to LPS in the human monocytic cell line THP-1. Journal of Clinical Biochemistry and Nutrition 44(1): 62–66. https://doi.org/10.3164/jcbn.08-182 [PubMed] [PMC]
- Schulz E, Gori T, Münzel T (2011) Oxidative stress and endothelial dysfunction in hypertension. Hypertension Research 34(6): 665–673. https://doi.org/10.1038/hr.2011.39 [PubMed]
- Sessa W (2011) MicroRNA regulation of cardiovascular functions. Arteriosclerosis, Thrombosis, and Vascular Biology 31(11): 2369. https://doi.org/10.1161/ATVBAHA.111.238311 [PubMed]
- Shapoval N, Obolenskaia O, Kalenikova E, Gorodetskaya E, Medvedev O (2018) Tissue distribution and redox status of coenzyme q10 after intravenous administration of ubiquinol to rat. Journal of Hypertension 36: e149–e150. https://doi.org/10.1097/01.hjh.0000539397.97203.46
- Sharma A, Fonarow G, Butler J, Ezekowitz J, Felker G (2016) Coenzyme Q10 and heart failure: A state-of-the-art review. Circulation Heart Failure 9(4): e002639. https://doi.org/10.1161/CIRCHEARTFAILURE.115.002639 [PubMed]
- Sharp J, Farha S, Park M, Comhair S, Lundgrin E, Tang W, Bongard R, Merker M, Erzurum S (2014) Coenzyme Q supplementation in pulmonary arterial hypertension. Redox Biology 2: 884–891. https://doi.org/10.1016/j.redox.2014.06.010 [PubMed] [PMC]
- Silva B, Pernomian L, Bendhack L (2012) Contribution of oxidative stress to endothelial dysfunction in hypertension. Frontiers in Physiology 3: 441. https://doi.org/10.3389/fphys.2012.00441 [PubMed]
- Steiner M, Syrkina O, Kolliputi N, Mark E, Hales C, Waxman A (2009) Interleukin-6 overexpression induces pulmonary hypertension. Circulation Research 104(2): 236–244. https://doi.org/10.1161/CIRCRESAHA.108.182014 [PubMed] [PMC]
- Thenappan T, Ormiston M, Ryan J, Archer S (2018) Pulmonary arterial hypertension: pathogenesis and clinical management. British Medical Journal 360: j5492. https://doi.org/10.1136/bmj.j5492 [PubMed]
- Tsai KL, Huang YH, Kao CL, Yang DM, Lee HC, Chou HY, Chen YC, Chiou GY, Chen LH, Yang YP, Chiu TH, Tsai CS, Ou HC, Chiou SH (2012) A novel mechanism of coenzyme Q10 protects against human endothelial cells from oxidative stress-induced injury by modulating NO-related pathways. Journal of Nutritional Biochemistry 23(5): 458–468. https://doi.org/10.1016/j.jnutbio.2011.01.011 [PubMed]
- Vásquez-Vivar J, Kalyanaraman B, Martásek P, Hogg N, Masters BS, Karoui H, Tordo P, Pritchard K (1998) Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proceedings of the National Academy of Science of the United

States of America 95(16): 9220–9225. https://doi.org/10.1073/pnas.95.16.9220 [PubMed] [PMC]

- Wang Q, Zuo X, Wang Y, Xie W, Wang H, Zhang M (2013) Monocrotaline-induced pulmonary arterial hypertension is attenuated by TNF-α antagonists via the suppression of TNF-α expression and NF-κB pathway in rats. Vascular Pharmacology 58(1–2): 71–77. https://doi.org/10.1016/j.vph.2012.07.006 [PubMed]
- Wharton J, Strange J, Møller G, Growcott E, Ren X, Franklyn A, Phillips S, Wilkins M (2005) Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. American Journal of Respiratory and Critical Care Medicine 172: 105–113. https://doi.org/10.1164/rccm.200411-1587OC [PubMed]
- Xu W, Erzurum S (2011) Endothelial cell energy metabolism, proliferation, and apoptosis in pulmonary hypertension. Comprehensive Physiology 1(1): 357–372. https://doi.org/10.1002/cphy.c090005 [PubMed] [PMC]
- Zhai J, Bo Y, Lu Y, Liu C, Zhang L (2017) Effects of coenzyme Q10 on markers of inflammation: A systematic review and meta-analysis. PLoS ONE 12(1): e0170172. https://doi.org/10.1371/journal.pone.0170172 [PubMed] [PMC]
- Zhou G, Chen T, Raj J (2015) MicroRNAs in pulmonary arterial hypertension. American Journal of Respiratory Cell and Molecular Biology 52(2): 139–151. https://doi.org/10.1165/rcmb.2014-0166TR [PubMed] [PMC]

Author contributions

- **Tatyana A. Kuropatkina,** PhD student, Lomonosov Moscow State University, School of Medicine, Department of Pharmacology, e-mail: 0sylphide0@gmail.com, **ORCID ID** http://orcid.org/0000-0003-1027-8703. Data collection and analysis, drafting and reviewing the manuscript.

- **Nadezda V. Pankova,** PhD, junior research associate, Institute of Biomedical Problems, Russian Academy of Sciences, e-mail: nadezhda.pankova.nnov@gmail.com, **ORCID ID** http://orcid.org/0000-0001-7474-3665. Data collection and analysis, drafting and reviewing the manuscript.

- **Natalia A. Medvedeva,** PhD, professor, Lomonosov Moscow State University, Faculty of Biology, Human and Animal Physiology, e-mail: namedved@gmail.com, **ORCID ID** http://orcid.org/0000-0001-5998-6781. Conception and design, data analysis, drafting and reviewing the manuscript.

- **Oleg S. Medvedev,** MD, PhD, professor, Lomonosov Moscow State University, School of Medicine, Chair of Pharmacology, e-mail: oleg.omedvedev@gmail.com, **ORCID ID** http://orcid.org/0000-0001-8942-4851. Conception and design, data analysis, drafting and reviewing the manuscript.