doi:10.25143/prom-rsu_2023-24_dts

Dana Kigitovica

Effects of Meldonium on Right Ventricular Failure in a Preclinical Model and Patients

Summary of the Doctoral Thesis for obtaining the scientific degree “Doctor of Science (*PhD*)”

Sector Group – Medical and Health Sciences
Sector – Clinical Medicine
Sub-Sector – Internal Medicine

Riga, 2023

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Defence of the Doctoral Thesis will take place at the public session of the Promotion Council of Clinical Medicine on 20 December 2023 at 14.00 in the Hippocrates Lecture Theatre, 16 Dzirciema Street, Rīga Stradiņš University and remotely via online platform *Zoom*

The Doctoral Thesis is available in RSU Library and on RSU website: https://www.rsu.lv/en/dissertations

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projekts
NACIONĀLAIS ATTĪSTĪBAS PLĀNS 2020
EIROPAS SAVIENĪBA
Eiropas Sociālais fonds
IEGULDĪJUMS TAVĀ NĀKOTNĒ

Research project was supported by the European Social Fund and Latvian state budget within project No. 8.2.2.0/20/I/004
"Support for involving doctoral students in scientific research and studies"
at Rīga Stradiņš University.

RĪGA STRADIŅŠ UNIVERSITY

Research project was supported by RSU *PhD* grant.

VPP
Valsts pētījumu programma

Research project was supported by the Latvian State Research Program project VPP-COVID-2020/1-0014
"Towards new therapeutic and prophylactic treatments
against Covid-19 and coronaviruses".

FLPP
FUNDAMENTAL AND APPLIED RESEARCH PROJECTS

Research project was supported by the Fundamental and Applied Reseach Projects lzp-2020/1-0055
"Implementation of balloon pulmonary angioplasty and evaluation of its effectiveness in treatment of chronic thromboembolic pulmonary hypertension at Pauls Stradins Clinical University Hospital".

Table of Contents

Abbreviations used in the Thesis

6MWT	the six-minute walk test
ATP	adenosine triphosphate
BDS	Borg dyspnoea score
BMI	body mass index
BNP	brain natriuretic peptide
CHF	chronic heart failure
CI	cardiac index
CO	cardiac output
CPT1	carnitine palmitoyltransferase-1
CTEPH	chronic thromboembolic pulmonary hypertension
FA	fatty acid
FAC	fractional area change
FAO	fatty acid oxidation
FC	functional class
Fio2	fraction of inspired oxygen
IPAH	Idiopathic pulmonary arterial hypertension
LV	left ventricle
MCS	mental component score
MCT	monocrotaline
mPAP	mean pulmonary arterial pressure
NYHA	New York Heart Association
PAH	pulmonary arterial hypertension
PCS	physical component score
PCWP	pulmonary capillary wedge pressure
PH	pulmonary hypertension
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator 1 alpha

PPARα	peroxisome proliferator activated receptor-alpha
PVR	pulmonary vascular resistance
QoL	quality of life
RAP	right atrial pressure
RV	right ventricle
RVF	right ventricular failure
SF-36	Short Form-36

Introduction

Right ventricular (RV) failure (RVF) is a clinical syndrome characterised by reduced right ventricular function leading to suboptimal blood delivery to the pulmonary circulation and/or elevated venous pressure at rest or during exercise (Mehra et al., 2014; Galiè et al., 2019). The reduced life expectancy (Voelkel et al., 2006) and poor quality of life (Winter et al., 2008) of RVF patients reflect the severity and clinical importance of the syndrome. Recently, there has been an increase in the number of publications emphasising the significance of right ventricular failure and the urgent need for the targeted treatment of RVF (Voelkel et al., 2006; Houston, Brittain, and Tedford, 2023). There are multivariate causes of RVF, such as left ventricular failure, chronic lung diseases, cardiomyopathies, right ventricular infarction, valvular diseases, pulmonary hypertension etc. (Voelkel et al., 2006). Pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are two rare pulmonary hypertension (PH) diseases that induce RVF, and early diagnosis plays a crucial role in initiating treatment for PH and preventing the progression to RVF.

Therapy for RVF is based on 3 options: reducing afterload, optimising preload, and increasing contractility (Lahm et al., 2010; Houston, Brittain, and Tedford, 2023). Currently, there are no specific drugs for the treatment of RVF (Prisco, Thenappan, and Prins, 2020). In addition, drugs used to treat conditions that induce RVF may attenuate the development of RVF but do not improve the right ventricular function (Prisco, Thenappan, and Prins, 2020). Specifically, etiological therapy for pulmonary arterial hypertension (PAH) targets the pulmonary circulation and reduces blood pressure in the pulmonary arteries, but has little or no effect on RVF (Ren, Johns, and Gao, 2019).

The development of RVF is characterised by altered myocardial energy metabolism, and improving mitochondrial function in experimental models of RV dysfunction has been associated with improved ventricular function (Sun et al., 2016; Fowler et al., 2019). To date, some metabolic modulators have shown some promising results in experimental RVF models (Guarnieri and Muscari, 1990; Guarnieri and Muscari, 1988; Archer et al., 2013; Fang et al., 2012; Prins et al., 2019; Paffett, Lucas, and Campen, 2012; Ren, Johns, and Gao, 2019). However, few of them improved quality of life and cardiac function in RVF patients (Khan et al., 2015; Han et al., 2021; NCT03273387, 2017).

Meldonium is a clinically used cardioprotective drug that reduces the levels of L-carnitine and its fatty acid ester, acylcarnitine levels, thereby modulating energy metabolism by preventing mitochondrial overload of fatty acid (FA) metabolism intermediates and redirecting FA flux to peroxisomal metabolism (Dambrova et al., 2016; Liepinsh et al., 2013). The addition of meldonium to existing treatment in patients with LV heart failure improved their quality of life and functional class (Dzerve et al., 2010), improved chronic heart failure (CHF) outcomes (Dzerve et al., 2005), and induced favourable changes in cardiac structural and functional parameters (Statsenko, Shilina, and Turkina, 2014).

So far, there is no information on the effects of meldonium on ventricular function and mitochondrial energetics in right ventricular failure, nor on the quality of life of RVF patients. The thesis is divided into three sections: 1) analysis of the incidence of PAH and CTEPH patients, 2) evaluation of the effects of meldonium on the right ventricle in the preclinical model of RVF, 3) the assessment of the clinical effects of meldonium in patients with RVF.

Aim of the Thesis

The study was conducted to analyse the incidence of two RVF-inducing diseases, PAH and CTEPH, in the Latvian population and to assess the effects of meldonium in preclinical model of RVF and in RVF patients.

Objectives of the Thesis

1. To analyse the incidence and characteristics of PAH and CTEPH patients in the Latvian population
2. To assess the effects of meldonium on experimentally induced RVF in a preclinical model
3. To assess the safety and clinical efficacy of meldonium in patients with PAH-induced RVF.

Hypotheses of the Thesis

Meldonium enhances the mitochondrial bioenergetics in the cardiomyocytes of the right ventricle and improves RV function and overall physical well-being in both experimental model rats and RVF patients.

Novelty of the Thesis

A major burden in the management of rare diseases, including PAH and CTEPH, is the lack data. A significant majority of patients with both PAH and CTEPH are typically diagnosed at an advanced stage of the disease, adding to the complexity of treatment. More consistent data on the baseline characteristics of PAH and CTEPH patients could contribute to a better understanding of these diseases. Initiating specific treatment at an earlier stage could help preserve RV function and reduce the pressure overload.

Currently, the RV therapy is based on symptomatic relief and treatment of the primary disease, but there is a lack of cardiospecific treatment focused on RV dysfunction and progressive RVF. Novel therapeutic approaches are essential in the treatment of the RV. The dysregulation of energy metabolism, including mitochondrial dysfunction, is a key component of RV progression to failure. We are the first to show that the treatment with meldonium attenuates the development of pulmonary arterial hypertension-induced RVF. Moreover, our results are the first to show that the stimulation and restoration of decreased mitochondrial FA metabolism in the right ventricle is capable of improving the function of the ventricle.

The conducted clinical trial demonstrates that treatment with meldonium significantly increases daily exercise capacity and reduces objective and subjective shortness of breath in patients with chronic RVF due to PAH. Our study demonstrates that meldonium is safe in patients with chronic RVF. No serious adverse events were observed during the 60-day treatment period. Overall, the results of the research bring to the forefront a promising cardiometabolic treatment option and drug for the management of RVF, offering hope to patients with progressive RVF.

Practical significance of the work

Our preclinical results show that meldonium improves RV function by modifying energy metabolism in the myocardium. The results of the clinical study indicate that meldonium may be a viable novel drug treatment to improve the quality of life (QoL) and functional capacity, and reduce dyspnoea in patients with chronic RVF, making it suitable for people with RVF.

1 Methods

The thesis is structured into three sections, which are presented in Figure 1.1.

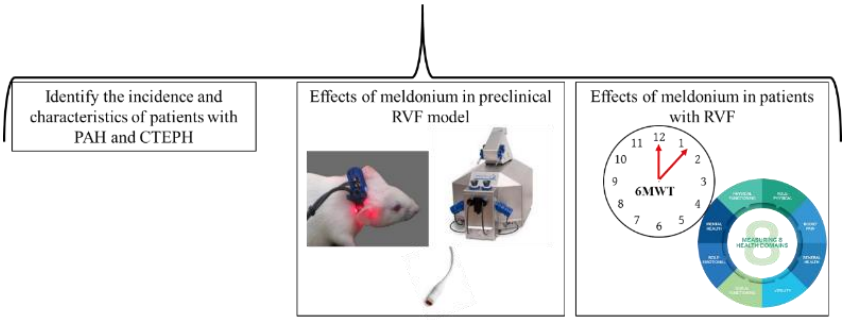

Figure 1.1 **Schematic representation of the thesis structure**

The visual representation partly adapted from: https://www.qualitymetric.com/health-surveys/the-sf-36v2-health-survey/

1.1 PAH and CTEPH incidence in Latvia

Between January 1 and December 31 of 2021, a prospective observational study conducted at Pauls Stradins Clinical University Hospital in Riga examined the Latvian Pulmonary Hypertension (PH) registry. Ethical approval was obtained from the Pauls Stradins Clinical University Hospital (Atzinums Nr. 250123-12L) before the initiation of the study. The study included a total of 18 patients with pulmonary arterial hypertension (PAH) and 8 patients with chronic thromboembolic pulmonary hypertension (CTEPH). Patients were included in the study based on diagnostic criteria consistent with the 6th World Symposium on Pulmonary Hypertension Task Force (Condon et al., 2019). To confirm the diagnosis of PH the right heart catheterization was performed in all patients, which measured the following haemodynamic parameters: RAP,

mPAP, pulmonary capillary wedge pressure (PCWP), PVR, CO and cardiac index (CI).

1.2 Effects of meldonium in preclinical RVF model

Eight-week-old 100 male Sprague-Dawley rats were obtained from Charles River Laboratories (Sulzfeld, Germany). Animals were housed in individually ventilated cages (three rats per cage) with unlimited access to food (R70 diet, Lantmännen Lantbruk, Sweden) and water. Standard housing conditions (temperature of 21–23 °C, 12-hour light/dark cycle and relative humidity of 50 ± 5 %). Rats were adapted to these housing conditions for at least one week before the beginning of the experiments. The experimental procedures were performed in accordance with the guidelines of the European Community as well as local laws and policies, and the procedures were approved by the Latvian Animal Protection Ethical Committee of the Food and Veterinary Service, Riga, Latvia. All studies involving animals were reported in accordance with the ARRIVE guidelines (Kilkenny et al., 2010; McGrath et al., 2010).

To study the effects of meldonium on RVF development, 34 rats were used. To study the effects of meldonium on the development of endothelial dysfunction in pulmonary arteries, 36 rats were used and to study the effects of meldonium on blood oxygen saturation 30 rats were used. All experimental procedures and analyses were performed by a scientific staff blinded to the treatment groups and the experimental groups were uncovered only after summarizing results.

Pulmonary hypertension and RVF in rats were induced by a single subcutaneous injection of monocrotaline (MCT) at a dose of 60 mg/kg in 24 animals. Control group rats (n = 10) received an injection of an equal volume of saline. Rats that received MCT were randomly allocated to two equal groups (n = 12). The animals in the MCT group continued to receive purified drinking

water, while the rats in the MCT + Meldonium group started to receive meldonium at a dose of 200 mg/kg/day together with purified drinking water for four weeks (Figure 1.). After the administration of MCT or vehicle, the weight of the animals was monitored twice per week.

Echocardiography was performed using Philips iE33 ultrasonograph (Philips Healthcare, Andover, MA) 28 days after the administration of MCT as described previously (Videja et al., 2021) with slight modifications. After 4 weeks of treatment, the direct measurement of systolic RV pressure was done. The vascular reactivity was evaluated in isolated pulmonary arteries using method described previously (Nakazawa et al., 1999; Mathew et al., 1995) with minor adjustments. Blood oxygen saturation (SpO2), respiratory rate and heart rate were measured before the administration of saline or MCT and once every week for 4 weeks using a pulse oximeter (MouseOx® Plus Pulse Oximeter for Rodents). The rats were euthanized, and the pulmonary and cardiac tissues were harvested for histological analysis and assessment of mitochondrial functionality. Mitochondrial function was assessed in permeabilized cardiac fibres that had been prepared as previously described (Kuka et al., 2012). Mitochondrial functionality measurements were evaluated using a previously described protocol (Videja et al., 2021; Makrecka-Kuka et al., 2020). The heart was cut out and divided into the right ventricle and the left ventricle with the septum. The lungs were collected and weighed to calculate the lung-to-body weight index and prepared for histological analysis to visually assess the extent of fibrosis.

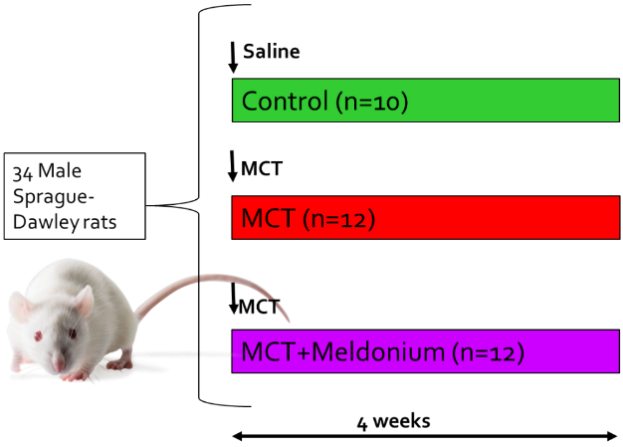

Figure 1.2 **Schematic representation of the study design**

1.3 Effects of meldonium in patients with RVF

This was an investigator-initiated observational study conducted in P. Stradins Clinical University Hospital, Riga, Latvia. Patients ≥ 18 years of age who fulfilled the criteria for RVF due to Group 1 PAH and were classified as World Health Organization (WHO) FC I to III were enrolled in the study. Each patient with symptomatic PAH belonged to one of the following 2018 Clinical Group 1 subtypes: idiopathic PAH (IPAH), heritable PAH, or PAH associated with connective tissue disease (CTD PAH). Written informed consent was obtained from all patients before the study. Ethical approval was obtained from the Pauls Stradins Clinical University Hospital (Atzinums Nr. 030221-8L) and the State Agency of Medicines of the Republic of Latvia (16.03.2021) before the initiation of the study. All experiments were performed in accordance with the State Agency of Medicines of the Republic of Latvia. Meldonium was provided by JSC Grindeks without any financial support during the study.

The study consisted of an initial visit, a safety control visit after 14 days, an examination of the patient after 30 days of treatment ± 7 days, and a health checkup after a washout period of 30 days ± 7 days. The study procedures included a clinical visit with a physical examination; completion of the SF-36; assessment of the Borg dyspnoea score (BDS); administration of the 6MWT; laboratory testing, such as full blood count, liver and kidney function tests and B-type natriuretic peptide (BNP); assessment of WHO FC; and determination of adverse events. The 6MWT was performed as described in the guidelines (Crapo et al., 2002). Study participants completed a quality-of-life questionnaire (SF-36) at three time-points: before treatment, just after treatment, and one month after the end of treatment. In addition, at the same time points, all participants underwent a standardized 6MWT, BDS evaluation and assessment of WHO FC; laboratory testing, however, was performed only at visit 3 (Figure 1.3).

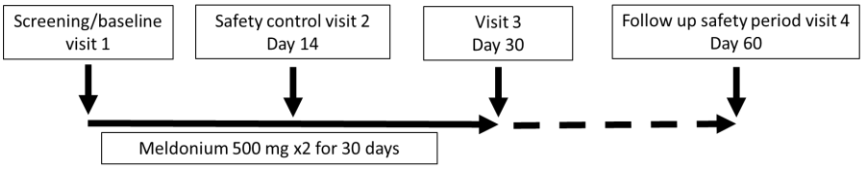

Figure 1.3 **Study schematic**

Right heart catheterization (RHC) was completed prior to the study in all patients to confirm the diagnosis of PAH according to the 6th World Symposium on Pulmonary Hypertension Task Force criteria. Patients were on a stable treatment regimen with one or more treatments approved for primary disease and CHF. Stable therapy was defined as constant therapy for ≥ 12 weeks before the screening visit and a stable dosage of each medication for ≥ 8 weeks before the screening visit. Patients remained on their previously prescribed background

medications for PH, heart failure and comorbidities without changing the dosage during the study period.

During the initial visit, patients received 60 meldonium (500 mg) capsules, which they had to take orally twice a day for the next 30 days. The treatment period with meldonium was selected based on the guidelines set by the State Agency of Medicines of the Republic of Latvia. The next follow-up visits were scheduled one month and two months after the first visit. During the study, the occurrence of serious adverse effects was assessed. Serious adverse events were defined as a fatal or serious deterioration of health resulting in death, risk of death, hospitalization for > 24 hours, disability or incapacitation, or intervention to prevent a life-threatening condition.

2 Statistical analysis

Continuous variables in the PAH and CTEPH incidence study were presented as the mean ± standard deviation, while categorical variables were reported as counts and percentages. Statistical analysis was performed using SPSS version 22.0 (IBM Corp., USA).

Preclinical data were expressed as the means ± standard error of mean (SEM). Shapiro-Wilk normality test was used to assess the data distribution. One-way ANOVA with Dunnett's multiple comparisons test was used for data that were distributed normally. Kruskal-Wallis with Dunn's multiple comparison test was used for cases where the data were not distributed normally. Two-way repeated measures ANOVA with Tukey's multiple comparison test was used to compare the differences in weight gain, vascular reactivity and blood oxygen saturation between the experimental groups. P values < 0.05 were considered to indicate statistical significance. The statistical calculations and the creation of the figures were performed using GraphPad Prism software.

Continuous variables from the clinical study were expressed as the mean ± standard deviation. Categorical variables are displayed as counts and percentages. Differences in SF-36 scores, 6-minute walk distance, and laboratory parameter assessments were tested using one of two significance tests for continuous variables: the dependent-samples t test or the nonparametric Wilcoxon test. A p value < 0.05 was considered significant. Statistical analysis was performed using SPSS version 22.0 (IBM Corp., USA).

3 Results

3.1 Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia

The female sex was predominant in the PAH patient group (72 % in PAH and 50 % in CTEPH group). A notable portion of enrolled patients showed a decreased functional capacity (low 6-min walking test and high proportion of NYHA class III). The increased haemodynamic parameters and the decreased functional capacity for the PAH and the CTEPH patients indicate of a severe progression of the disease at the time of the diagnosis. The detailed baseline characteristics are shown in Table 3.1.

Table 3.1

Baseline characteristics of Latvian PAH and CTEPH patients

Parameter	PAH	CTEPH
Number of patients, n	18	8
Age, years	71.7 ± 12.2	72.8 ± 8.0
Females, n (%)	13 (72)	4 (50)
BMI, kg/m2	29.8 ± 6.1	27.3 ± 5.1
6 MWT, m	255.2 ± 100.1	296.6 ± 155.3
NYHA class I-II/III/IV, %	44.4/44.4/11.2	37.5/37.5/25
RAP, mmHg	5.8 ± 5.2	7.3 ± 5.8
RVSP, mmHg	65.7 ± 22.6	72.6 ± 13.6
mPAP, mmHg	38.8 ± 15.2	45.1 ± 8.9
PCWP, mmHg	10.6 ± 5.7	6.7 ± 3.6
PVR, Wood units	8.1 ± 4.6	8.6 ± 3.3
CO, l/min	4.1 ± 1.3	4.7 ± 0.9
CI, l/min/m2	2.3 ± 0.7	2.5 ± 0.4
BNP, pg/ml	359.9 ± 248.3	607.7 ± 421.7

Values are shown as frequencies and proportions (%) or mean ± standard deviation. CI – cardiac index; CO – cardiac output; BMI – body mass index; BNP – B-type Natriuretic Peptide; mPAP – mean pulmonary artery pressure; NYHA – New York Heart Association; PCWP – pulmonary capillary wedge pressure; PVR – pulmonary vascular resistance; RAP – right atrial pressure; RVSP – right ventricular systolic pressure; 6MWT – 6-min walking test.

The estimated incidence of PAH in 2021 was 9.5 per million inhabitants, 11.7 per million adult population, respectively; whereas the incidence of CTEPH was 4.2 per million inhabitants, 5.0 per million adult population, respectively. The incidence of the PAH and the CTEPH patients included in the Latvian PH registry (data for the past five years) is summarized in Table 3.2.

Table 3.2

The last 5-year incidence of PAH and CTEPH patients from Latvian Pulmonary hypertension registry

	2021	**2020**	**2019**	**2018**	**2017**
PAH incidence (per million residents)	9.5	12.6	11.4	7.2	9.2
PAH incidence (per million adult residents)	11.7	15.5	14.1	9.0	11.3
CTEPH incidence (per million residents)	4.2	3.7	3.6	3.6	11.8
CTEPH incidence (per million adult residents)	5.0	4.5	4.5	4.5	14.5

Values are shown as frequencies. CTEPH – chronic thromboembolic pulmonary hypertension; PAH – pulmonary arterial hypertension. Table is published in: (Kigitovica et al., 2019).

3.2 Effects of meldonium in preclinical RVF model

3.2.1 Overall animal well-being

The health of the animals was monitored every day, and none of the animals died during the four-week treatment. The weight gain of the animals that received MCT was slower than that of the control group animals (Figure 3.1); however, the weight gain in the MCT + Meldonium group in the final week of the experiment was significantly faster than that in the MCT group. During the 28-day observation period, the weight gain in the animals in the control, MCT and MCT + Meldonium groups was 86 ± 5, 26 ± 7 and 47 ± 4 g, respectively.

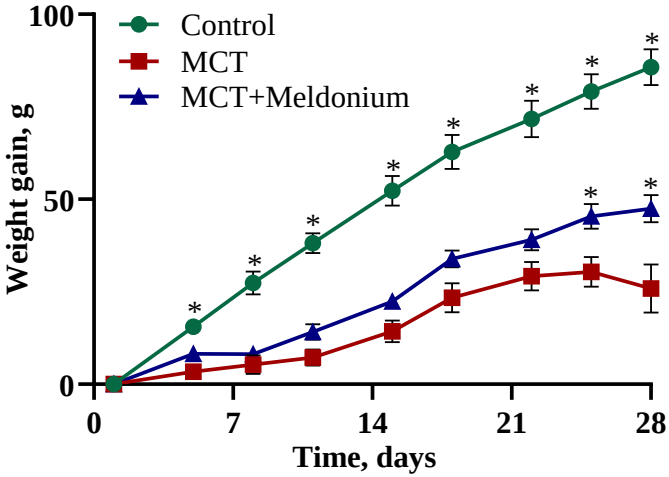

Figure 3.1 **Effects of treatment with meldonium on the weight gain of the animals**

Rats receiving meldonium gained weight significantly faster than animals from the MCT group. The results are shown as the mean ± SEM of 10-12 animals. *p < 0.05 vs. MCT group, two-way repeated measures ANOVA with Tukey's multiple comparisons test. MCT – monocrotaline

3.2.2 Effects of meldonium on ventricular size and function in the right ventricular failure model

RV systolic pressure was significantly increased in the MCT group compared to the control group, and treatment with meldonium did not attenuate the elevation in RV systolic pressure (Table 3.3). Administration of MCT induced the development of RVH, which was evident by an elevated right ventricle-to-body mass index and Fulton index (right ventricle/ (left ventricle + septum)). Treatment with meldonium attenuated the development of RVH. As shown in Table 3.3, the administration of MCT did not increase the left ventricle-to-body mass index, and the size of the left ventricle was not influenced by treatment with meldonium.

Table 3.3

Effects of meldonium administration on RV pressure, right ventricle-to-body, right ventricle-to-left ventricle and left ventricle-to-body weight indexes

	Control	MCT	MCT + Meldonium
RV systolic pressure, mmHg	19 ± 1*	52 ± 5	41 ± 4
RV-to-body mass index, mg/g	0.50 ± 0.01*	1.13 ± 0.06	0.88 ± 0.08*
Fulton index, g/g	0.27 ± 0.01*	0.53 ± 0.03	0.43 ± 0.04*
LV-to-body mass index, mg/g	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1

Treatment with meldonium significantly attenuated the development of RV hypertrophy and had no effect on RV pressure. The results are shown as the mean ± SEM of 10 to 12 animals. *$p < 0.05$ vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. LV – left ventricle, MCT – monocrotaline, RV – right ventricle

The analysis of echocardiographic parameters revealed that administration of MCT significantly increased the end-diastolic area (EDA) and end-systolic area (ESA) of the right ventricle (Figure 3.2 A, B). Treatment with meldonium attenuated the development of dilatation of the right ventricle and significantly decreased ESA; moreover, the results showed a tendency of meldonium treatment to decrease the EDA compared with that of the MCT group. The EDAs in the control, MCT and MCT + Meldonium groups were 0.5 ± 0.02, 0.9 ± 0.02 and 0.7 ± 0.07 cm^2, respectively. The ESAs in the control, MCT and MCT + Meldonium groups were 0.3 ± 0.02, 0.7 ± 0.06 and 0.5 ± 0.06 cm^2, respectively (Figure 3.2 A, B). In addition, MCT administration significantly decreased the RV fractional area change (RVFAC) by 42 %. Four weeks of treatment with meldonium significantly improved the functioning of the right ventricle and increased RVFAC by 40 % compared with the MCT group (Figure 3.2 C).

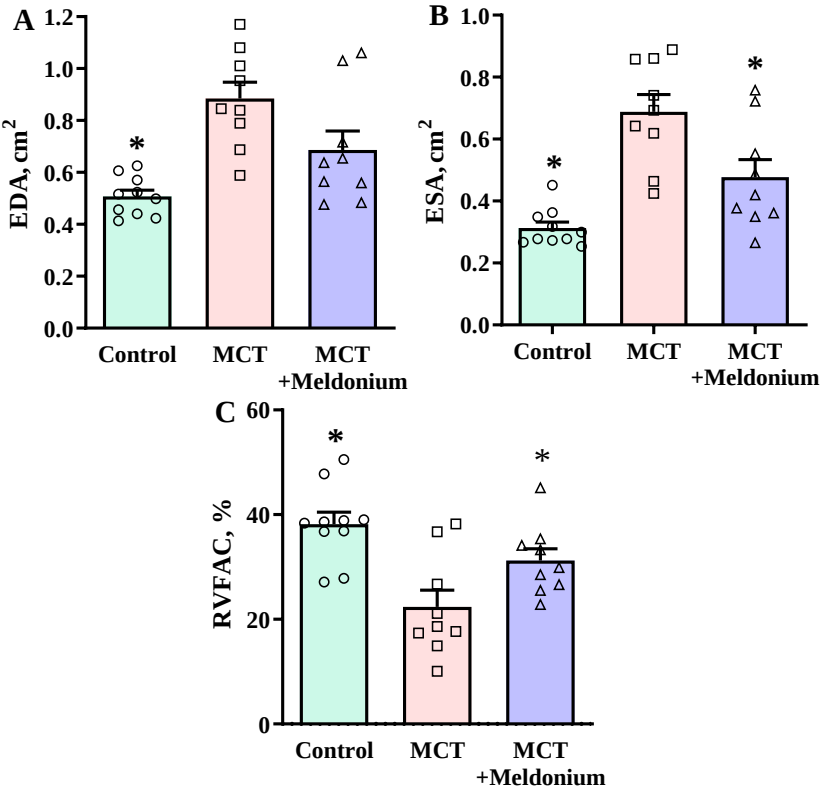

Figure 3.2 **Effects of meldonium administration on the (A) end-diastolic area (EDA) of the right ventricle, (B) end-systolic area (ESA) of the right ventricle and (C) fractional area change of the right ventricle (RVFAC)**

Treatment with meldonium significantly decreased ESA and increased RVFAC compared with the MCT group. The data are shown as the mean ± SEM of 7 to 10 animals. *p < 0.05 vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. MCT – monocrotaline

3.2.3 Effects of meldonium on mitochondrial function in the right ventricular failure model

In the MCT group, FAO (F(N)) pathway-dependent respiration in the OXPHOS state was significantly decreased, by 46 % (Figure 3.3 A), which resulted in a 23 % decrease in FAO-dependent OXPHOS coupling efficiency

(Figure 3.3 B) compared with that in the control group. Moreover, despite stimulation of pyruvate metabolism, as indicated by flux control factor analysis (Figure 3.3 B), MCT administration decreased the FN and FNS pathway-linked respiration rates in the OXPHOS state (Figure 3.3 A). In addition, in the MCT group, partial dysfunction of complex I was observed, as indicated by a decrease in flux control factor upon rotenone treatment (Figure 3.3 B). Treatment with meldonium restored FAO-dependent OXPHOS coupling efficiency and subsequently decreased pyruvate metabolism and prevented complex I dysfunction (Figure 3.3 B). As a result, F(N), FN and FNS pathway-linked respiration in the OXPHOS state was improved in the MCT + Meldonium group (Figure 3.3 A). These results show that meldonium treatment normalizes mitochondrial function in the heart in conditions of pulmonary hypertension and RVF.

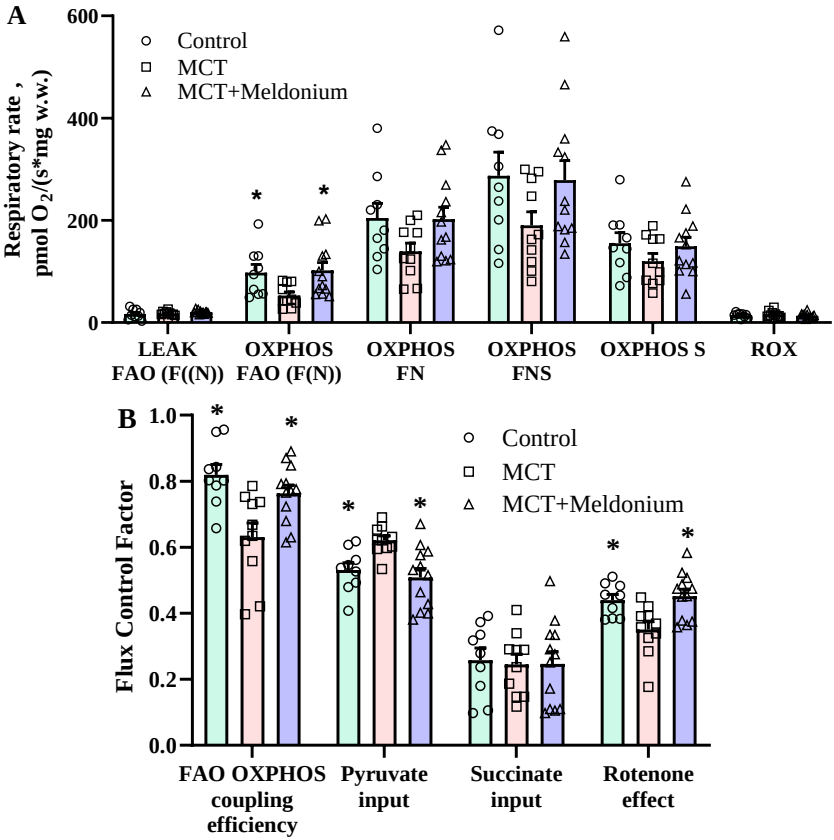

Figure 3.3 **The effects of meldonium treatment (200 mg/kg for 4 weeks) on the mitochondrial respiration rate (A) and flux control factors (B) in RV cardiac fibres 4 weeks after MCT injection**

MCT administration induced inhibition of FA-dependent oxidative phosphorylation, stimulation of pyruvate metabolism and partial complex I dysfunction. Treatment with meldonium restored mitochondrial functionality in the heart. The results are presented as the mean ± SEM of 9 to 12 animals. *$p < 0.05$ vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. Flux control factor – the contribution of each substrate/pathway to the respiration rate, S – succinate, F – FA oxidation-dependent pathway, N – NADH pathway, LEAK – substrate-dependent state, MCT – monocrotaline, OXPHOS – oxidative phosphorylation-dependent state, ROX – residual oxygen consumption

3.3 Effects of meldonium in patients with RVF

A total of 22 patients who met the inclusion criteria were enrolled in the study from 2021 to 2022. Two patients refused to continue the study because they were unable to make onsite visits due to the COVID-19 pandemic. During the 2 months of the study, enrolled patients did not report any SAEs or other AEs. After 30 days, patients returned the dispensed meldonium pack. There were no instances of missed doses recorded. The mean age of the patients at the beginning of the study was 70.4 ± 13.2 years, the majority of patients (75 %) were female, and PAH was the primary disease – IPAH (n = 14) and CTD PAH (n = 6). The most common WHO FC at baseline was class III (65 %).

The analysis of the 6MWT results revealed that patients were able to walk significantly longer distances after meldonium treatment than before (Figure 4 A). Before treatment, patients were able to walk 352.2 ± 114.8 m, but after 30 days of meldonium treatment, the walking distance increased to 398.9 ± 128.5 m ($p = 0.021$). On day 60 of the study, the results from the 6MWT demonstrated that the walking distance returned to the pretreatment value (376.7 ± 113.8 m, $p > 0.05$). In addition, treatment with meldonium markedly decreased the BDS (Figure 3.4 B) from the baseline score of 5.4 ± 2.2 to 3.4 ± 2.5 at day 30 ($p = 0.003$), and the effect persisted at day 60 with a score of 3.7 ± 2.5 ($p = 0.004$) (Figure 4 B).

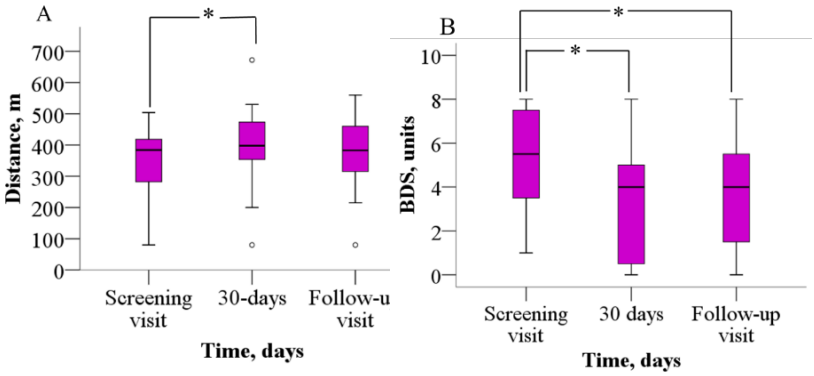

Figure 3.4 **The effect of meldonium on (A) 6MWT performance and (B) BDS**

The graphs represent the increase in 6MWT and decrease in BDS after treatment with meldonium. The results are shown as the mean ± SD of 20 patients; *p < 0.05 vs. the value as of the screening visit, paired-sample t test for 6MWT, Wilcoxon signed-rank test for BDS. BDS – Borg dyspnoea score, 6MWT – six-minute walk test

Thirteen study participants (65 %) reported advanced FC III symptoms at baseline, of whom 65 % improved to FC II symptoms (p = 0.031) by day 30, while the rest remained WHO FC III. After a 30-day washout period, 55 % of the patients were in WHO FC III (p > 0.05) (Figure 3.5).

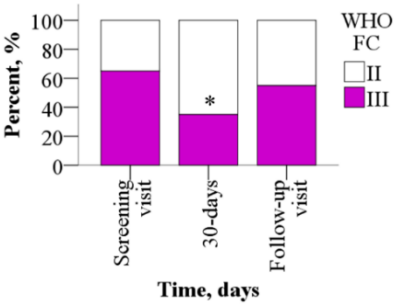

Figure 3.5 **WHO FC before and after treatment with meldonium**

Treatment with meldonium improved the functional class of the patients. The results are shown as percentages of 20 patients, *p < 0.05 vs. screening visit, chi-square test. WHO FC – World Health Organization functional class

The total SF-36 score increased after from 72.6 ± 17.7 points during the initial visit to 82.1 ± 14.8 points ($p = 0.009$) after 30 days of meldonium treatment. After the washout period, the SF-36 score decreased to 77.1 ± 17.7 points.

The mean mental component summary (MCS) of the SF-36 was 84.9 ± 15.6 points before the treatment, 87.7 ± 13.2 points ($p > 0.05$) after the 30-day treatment, and 83.6 ± 19.8 points at day 60 ($p > 0.05$). As shown in Figure 6, the physical component summary (PCS) score was 60.3 ± 23.1 points before treatment, but after 30 days and 60 days, scores decreased to 75.1 ± 17.6 and 70.7 ± 21.2 points, respectively ($p < 0.05$). Treatment with meldonium induced improvement in the Physical Functioning, Role–Physical and Bodily Pain domains of the SF-36.

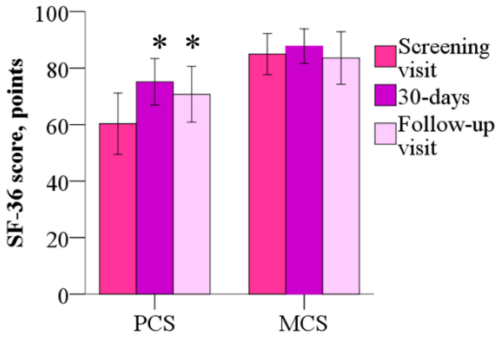

Figure 3.6 **The 36-Item Short Form Health Survey mental component summary and physical component summary before and after 30 days of treatment with meldonium**

Treatment with meldonium improved the functional capacity of the patients but had no effect on MCS. The results are shown as the mean of 20 patients; *$p < 0.05$ vs. PCS before the treatment, Wilcoxon signed-rank test. MCS – mental component summary; PCS – physical component summary

The MCS is composed of role limitations due to emotional problems, social functioning, emotional well-being, and energy/fatigue; none of these subscales showed improved scores after 30 days of treatment with meldonium or after 60 days from the beginning of the study. No changes were noted in the subscore for general health. PCS parameters such as physical functioning and bodily pain improved after 30 days and remained above the screening score at day 60 ($p < 0.05$), but role limitations due to physical health were improved only at day 30 (Table 3.4).

Table 3.4

SF-36 subscale scores at the baseline visit, after 30 days and at the follow-up visit

	Baseline measurement, points	**Measurement after 30 days, points**	**Measurement at follow-up, points**
Physical functioning	58.0 ± 25.9	71.0 ± 23.0*	67.8 ± 25.8*
Role limitations due to physical health	60.0 ± 42.5	85.0 ± 28.6*	73.8 ± 40.1
Role limitations due to emotional problems	90.0 ± 26.7	95.0 ± 16.3	88.3 ± 27.1
Energy/fatigue	76.5 ± 21.0	82.3 ± 15.2	76.0 ± 21.9
Emotional well-being	86.4 ± 15.9	86.2 ± 16.7	81.8 ± 17.1*
Social functioning	86.9 ± 21.6	92.5 ± 19.6	88.1 ± 23.5
Pain	67.5 ± 30.6	83.3 ± 24.1*	89.1 ± 16.7*
General health	55.8 ± 23.2	61.3 ± 20.5	52.3 ± 23.6

The results are shown as the mean ± SD of 20 patients. * $p < 0.05$ vs. baseline measurement, Wilcoxon signed-rank test

4 Discussion

The present work provides evidence about the incidence of PAH and CTEPH which are both diseases that can lead to RVF, as well as the beneficial effect of meldonium on RVF in both preclinical model and patients with RVF.

Our results reveal that the overall incidence of both RVF-inducing diseases in Latvia has remained stable over the past five years, and their incidence is comparable to that in other European countries. However, a significant portion of patients in Latvia are still being diagnosed at advanced stages of the disease, with more than 50 % of them classified as NYHA functional class III and IV.

The results of the present preclinical model study demonstrate that treatment with meldonium attenuated the development of pulmonary hypertension-induced RVF. Administration meldonium attenuated the development of RVH and increased RVFAC by 50 %. The improvement of ventricular function was attributed to the improved mitochondrial bioenergetics in the cardiomyocytes of the right ventricle. The positive effects of meldonium in preclinical study prompted the initiation of the clinical observational study involving patients with chronic RVF.

Our clinical results are the first to demonstrate that treatment with meldonium significantly increases daily physical performance and diminishes shortness of breath in patients with chronic RVF due to PAH. Meldonium treatment improved BDS and parameters characterizing objective and subjective physical functioning. At baseline, 65 % of patients in this study were in WHO FC class III, but after the 30-day meldonium treatment, WHO FC class III included only 35 % of the patients. In addition, our study demonstrates that meldonium is safe in patients with chronic right heart failure. No major AEs were observed during the 60-day period.

4.1 Global trends of the incidence of PAH and CTEPH

Nowadays, PAH is no longer considered a disease primarily affecting young females. Recent data reveal that the majority of the PAH patients are actually aged over 60 years at the time of diagnosis (Rådegran et al., 2016; Ling et al., 2012). Similar data are presented also from COMPERA registry data (Hoeper et al., 2013). In 2021, our data indicate that the average age at which PAH and CTEPH are diagnosed is 71 and 72 years, respectively. However, when examining the data from 2018, the average age at diagnosis for PAH and CTEPH was 65 and 64 years, respectively (Kigitovica et al., 2019). According to the PH registry data collected from September 1, 2007, to December 31, 2016, the mean age at the time of diagnosis for patients with PAH and CTEPH was 65 and 67 years, respectively (Skride et al., 2018). In all cases, a significant portion of patients were identified as being in an advanced stage of the disease, with over 50 % classified as NYHA functional class III and IV and low 6MWD (Kigitovica et al., 2019; Skride et al., 2018). The incidence of PAH and CPTEH has remained relatively stable since 2016 (Skride et al., 2018), however, there was unexpectedly high incidence of CTEPH noted in 2017. The estimated prevalence of PAH and CTEPH in Latvia in 2016 was 45.7 and 15.7 cases per million residents, respectively (Skride et al., 2018).

The global prevalence of PH is approximately 1 % with an increase up to 10 % in patients aged 65 and above. The leading causes contributing to PH are left-sided heart and lung diseases (Hoeper et al., 2016). Between 2010 and 2020, the national PH unit in Ireland recorded a total of 163 cases of PAH with the annual incidence of PAH to be 3.11 cases per million population. The annual incidence of the idiopathic PAH was estimated 0.63 cases per million (Cullivan et al., 2022). The annual incidence of PAH in other European countries vary from 1.5 to 10.7, with the minimum value in Portugal and maximal incidence in Czech republic (Peacock et al., 2007; Billings et al., 2019; Humbert et al., 2006;

Escribano-Subias et al., 2012; Jansa et al., 2014; Gall et al., 2017; Hoeper, Huscher, and Pittrow, 2016; Korsholm et al., 2015; Baptista et al., 2013). In general, the incidence in Latvia is comparable to that of other European countries currently. The peak was between 2007 and 2016 with an estimated incidence of idiopathic PAH to be 7.6 cases a year (Skride et al., 2018). To sum up, PAH is a rare disease with a prevalence estimated around 10–50 cases per million inhabitants and an incidence of 5–10 cases per million per year (Lau et al., 2017). It is also noted that PAH affects predominantly females, and women have better right ventricular function that leads to favourable overall prognosis (Cheron et al., 2021).

4.2 Effects of meldonium on the development of right ventricular failure in MCT model

Right ventricular dysfunction can be prevented by decreasing etiological factors or by directly stimulating ventricular contractility. It has been demonstrated that attenuation of RV remodelling and improvement of RV function can be achieved by reducing the increase in pulmonary vascular resistance (Bhat et al., 2018) or by direct RV pharmacological stimulation by inotropic drugs (Tavares-Silva et al., 2017). In general, the available treatment choices are influenced by the underlying cause of the RV dysfunction and ineffective compensatory mechanisms (Houston, Brittain, and Tedford, 2023). Our results show that MCT administration induced RVH and increased its mass, but the treatment with meldonium decreased RV-to-body mass index and Fulton index which might indicating the reversibility or attenuation of the development of RVH. Treatment with meldonium improved the functioning of pulmonary hypertension-induced RVF; however, the enhanced contractility of the right ventricle was not related to endothelial function in pulmonary vessels, RV pressure or blood oxygen saturation. Thus, it can be concluded that meldonium

acts directly on the myocardium by modulating its energy metabolism and thus improving ventricular function. This result indicates that meldonium is suitable for combination treatments with drugs that decrease pulmonary vascular resistance to reduce RV remodelling and improve RV function, as well as in conjunction with drugs that reduce preload and afterload, and provide inotropic support.

The development of RVF is characterised by altered myocardial energy metabolism, such as downregulation of FA oxidation, altered oxidative metabolism and subsequent upregulation of glucose uptake and glycolysis (Koop et al., 2019). In our study, we observed cardiac FA oxidation disturbances and complex I partial dysfunction after the development of RVF (Vilskersts et al., 2022). FA metabolism disturbances in the failing myocardium of the right ventricle have been attributed to the downregulation of peroxisome proliferator activated receptor-alpha (PPARα)/PPAR-gamma coactivator-1alpha (PGC-1α) expression and decreased expression of several PGC-1α target genes encoding key enzymes that regulate FA oxidation (Gomez-Arroyo et al., 2013). Previously it was showed that treatment with meldonium activated the PPARα/PGC1α pathway, increased the expression of genes involved in FA metabolism and stimulated mitochondrial β-oxidation (Liepinsh et al., 2013). In the present study, treatment with meldonium restored FA oxidation-dependent OXPHOS coupling efficiency in fibres obtained from the right ventricle of hearts with RVF, which can be explained by activation of the PPARα/PGC1α pathway. In addition, treatment with meldonium decreased the accumulation of FA intermediates, thus facilitating the electron transfer system (Nouws et al., 2014; Liepinsh et al., 2016) and protecting mitochondrial function. It has been suggested that, at the mitochondrial level, loss of complex I assembly may be involved in the switching of energy metabolism to glycolysis (Rafikov et al., 2015). Another study proposed that the alterations in mitochondrial function observed in RVF can be

mainly attributed to complex I dysfunction (Wüst et al., 2016). Treatment with meldonium reversed complex I dysfunction in the fibres of the myocardium of the right ventricle and thus restored the functionality of the electron transfer system. Overall, meldonium treatment maintained the function of the right ventricle due to the preservation of FA metabolism and complex I function. The previous data showed that RVH is characterized by increased fatty acid oxidation (FAO) due to the elevated expression of carnitine palmitoyltransferase-1 (CPT-1) (Bruce et al., 2009; Singh et al., 2019). Meldonium due to its L-carnitine lowering effect reduce CPT-1 dependent mitochondrial FA oxidation that is compensated by an increase in peroxisomal FA metabolism (Liepinsh et al., 2013). Therefore, the summary FAO activity is not decreased and hypothetically this can lead to lower levels of toxic long-chain FFAs metabolism intermediates.

The protective effects of metabolic modulators on altered energy metabolism and the function of the right ventricle have been studied previously (Koop et al., 2019). Most of the previous studies have primarily focused on the activation of glucose metabolism resulting in the reduction of glycolysis. This process can be triggered by recoupling glycolysis with glucose oxidation due to inhibition of phosphorylation of the pyruvate dehydrogenase complex or indirectly by inhibition of FA metabolism (Koop et al., 2019). However, there is experimental evidence that in case of RVF fatty acids and various their metabolism intermediates accumulate in cardiomyocytes causing lipotoxicity (Talati and Hemnes, 2015). Thus, stimulating fatty acid metabolism should also be a promising mechanism to enhance ATP synthesis. Meldonium has previously demonstrated its effectiveness promoting the utilization of FA in mitochondria simultaneously redirecting them from mitochondria to peroxisomes (Dambrova et al., 2016). Our results show that treatment with meldonium restored FA oxidation-dependent OXPHOS coupling efficiency to the level of healthy controls and improved the function of the right ventricle. We are the first to show

that stimulation and restoration of decreased mitochondrial FA metabolism in the right ventricle is capable of improving the function of the ventricle. In contrast to stimulation of glucose oxidation, intensifying of FA oxidation would resemble more physiological energy metabolism in the right ventricle, as more than 70 % of the ATP in the myocardium of the healthy right ventricle is produced by FA metabolism (Fillmore, Mori, and Lopaschuk, 2014). Moreover, stimulation of fatty acid metabolism would decrease synthesis of lipotoxic substances. Taken together, the results demonstrate that the restoration of mitochondrial bioenergetics is sufficient to improve ventricular function.

To date, few drugs have been available to directly stimulate the function/contractility of a failing right ventricle (Konstam et al., 2018). Some clinical studies have shown that treatment with meldonium enhances the function of the left ventricle in heart failure patients (Statsenko, Shilina, and Turkina, 2014; Statsenko et al., 2007). This does not guarantee that meldonium treatment will improve the function of the right ventricle in patients as left and right ventricle in many aspects are not the same; however, our results show that administration of meldonium modifies energy metabolism in the myocardium of the right ventricle and, thus, can improve its function and decrease mortality in patients with RVF. The data obtained in preclinical setup lay grounds for the further clinical trials or observations to test whether similar effects are observed in RVF patients after meldonium treatment.

4.3 Impact of meldonium on patients with right ventricular failure

The 6MWT is a commonly used test for the objective assessment of functional exercise capacity for the management of patients with moderate to severe pulmonary disease and is especially widely used in patients with PH and RVF (Holland et al., 2014). 6MWT performance in PAH patients with RVF can

be increased by drugs that alter the function of the pulmonary vasculature. All currently used PAH treatment produce an observable increase in 6MWT performance from baseline to the endpoint of the study (Sommer et al., 2021). In addition, 24 weeks of treatment with the β-blocker nebivolol was found to increase 6MWT distance, induce a drop in BDS, and lower the FC of the patients (Martynyuk, Konosova, and Chazova, 2012). The effects of nebivolol can be attributed to the vasoprotective effects induced by its β2 and β3 agonist properties (Perros et al., 2015), as other classical β-blockers had no effect on 6MWT distance in RVF patients (Perros et al., 2017; Andersen et al., 2015). The results from a preclinical PAH-induced RVF model revealed that meldonium treatment had no effect on pulmonary vascular reactivity (Vilskersts et al., 2022). Thus, it can be concluded that the improvements induced by meldonium treatment are due to the modification of energy metabolism pathways. The functional status of RVF patients can also be improved by other drugs that modulate energy metabolism. In a study that administered ranolazine to PAH patients for a three-month period, there was an increase in 6MWT performance from 383 ± 60 m to 419 ± 80 m, along with a slight, statistically nonsignificant increase in the Kansas City Cardiomyopathy Questionnaire summary score (Khan et al., 2015). Similar findings were reported in a randomized double-blind placebo-controlled trial in PAH patients with trimetazidine: the trimetazidine-treated patients showed an improvement in functional capacity (NCT03273387, 2017). In our study, meldonium produced remarkable improvements in 6MWT performance (from 352 ± 115 to 399 ± 129 m) and in the physical component summary of the SF-36 after only 1 month of therapy. Moreover, some subscale SF-36 scores and BDS were significantly improved even after a 30-day washout period. This can be explained by the months-long elimination period of meldonium (Liepinsh and Dambrova, 2016; Rabin et al., 2019) and the prolonged presence of meldonium in tissues. In addition, two-thirds of the included patients

were in WHO FC III, with marked limitation of physical activity and correspondingly high BNP; nevertheless, meldonium therapy significantly lowered their WHO FC, which demonstrates that meldonium treatment improves conditions after a relatively short treatment period even in patients with severe disease.

The majority of studied patients continued to use more than one drug during the study and had various comorbidities. Meldonium treatment was started as an additional therapy, but nevertheless, it induced significant improvements in the context of other drugs and comorbidities without producing significant AEs during the treatment or follow-up period; thus, meldonium can be safely combined with other drugs used by RVF patients, and its efficacy may not be influenced by comorbidities. Hypothetically, the effects of meldonium may not be observed in patients who are already using metabolic modulators, as their energy metabolism has already changed; however, none of the participants in this study reported the use of any other metabolic modulator.

Our observational study shows that meldonium increases exercise capacity in patients with RVF. However, from the existing results, it is not possible to indicate whether the effects of meldonium were due to modified energy metabolism in the myocardium, the skeletal muscles, or both. The increase in 6MWT performance was not accompanied by tachycardia or blood pressure elevation, which indirectly demonstrates an increase in physical tolerance, as well as stable cardiac output. A previous clinical study showed that 12 months of meldonium therapy in patients with stable angina pectoris increased total exercise time and time to the onset of angina, which may indicate modified energy metabolism in the heart muscle (Dzerve et al., 2010). On the other hand, another study showed improvement of exercise tolerance with 24 weeks of meldonium treatment in patients with peripheral arterial disease and intermittent claudication (Dzerve et al., 2011). In the second clinical trial, the improvement

in exercise tolerance can be attributed to improved energy metabolism in skeletal muscles and their ability to perform better under partially ischemic conditions. In addition, as corroborated by some of the findings from the present study, exercise tolerance in patients with peripheral arterial disease was still improved one month after the discontinuation of meldonium therapy (Dzerve et al., 2011). More detailed studies are needed to understand the exact site of action of meldonium in patients with RVF.

QoL is a complex outcome that consists of an individual's satisfaction in the physical, social, and psychological domains; unfortunately, an improvement in objective physical functioning does not always lead to an improvement in QoL (Chen, Taichman, and Doyle, 2008; Halank et al., 2013; Rival et al., 2014). An improvement of SF-36 scores after the treatment, especially in physical functioning, might be associated with increased exercise capacity (Taichman et al., 2005). There is still a debate among various conclusions regarding whether the QoL score can predict mortality or deterioration of disease; however, Blok et al. showed that in PAH CHD patients, a decrease in SF-36 PCS is a determinant of mortality (Blok et al., 2015); Mathai et al. showed that SF-36 scores are associated with survival in patients with PAH (Mathai et al., 2016); and Johansson et al., in the Global Congestive Heart Failure Study, demonstrated that lower health-related QoL is associated with a higher risk of all unfavourable outcomes (Johansson et al., 2021). Jorge et al. compared QoL data among patients with and without heart failure, independent of the syndrome phenotype; they found significantly greater mean SF-36 scores in patients without HF than in those with HF, and the functional capacity of patients with HF was notably worse than that of patients without HF (Jorge et al., 2017). Therefore, we can hypothesize that treatment with meldonium positively influences SF-36 physical subscale scores toward those of the general population, which might mitigate HF patients' increased risk for mortality due to primary disease.

In 2011, the SF-36 was used to determine health-related QoL in the Latvian population (Ivanovs, Eksteina, and Viksna, 2011). The physical functioning, role–physical, bodily pain, and general health parameters were significantly decreased in the RVF group in comparison to the population data. Treatment with meldonium increased SF-36 subscale scores as it increased exercise capacity, and RVF patients were able to perform their everyday duties. The emotional parameters did not differ greatly from those of the general population; however, 55 % of the RVF patients were actively using psychopharmacological drugs that might improve their emotional well-being. After meldonium treatment, patients' SF-36 scores even more closely approached those of the overall population.

Limitations of this observational study include its relatively short timeframe (limited to 60 days), particularly bearing in mind the chronicity of the disease, as well as the lack of a placebo control. The observed findings in this study might be due to the composite effect of PAH-specific therapy and heart failure therapy received in addition to meldonium; on the other hand, the concomitant treatment was stable during the last 3 months with no augmentation of the functional parameters. Another limitation is the small sample size; however, the overall prevalence of the disease is low, and the patients, who were enrolled from the national PH registry, were representative of the average patient with RVF caused by PAH. Further clinical trials including a placebo group are needed to study the efficacy and safety of longer treatment periods, as well as to understand in more detail the mechanism and site of action of meldonium and its effects on RV function.

Conclusions

In summary, the obtained results confirm the proposed hypothesis that meldonium enhances the mitochondrial bioenergetics in the cardiomyocytes of the right ventricle and improves RV function as well as enhance overall physical well-being of RVF patients and animals with RVF.

1. The overall incidence of PAH and CTEPH in Latvia has remained stable over the past five years and it is comparable to that of other European countries. However, more than 50 % of patients are still diagnosed at advanced stages of the diseases.
2. In experimental RVF model, meldonium treatment prevents the development of RVF by improving mitochondrial bioenergetics. Modulators of mitochondrial energy metabolism could be a potential treatment option for RVF.
3. Meldonium treatment increases functional capacity and decreases dyspnoea in patients with chronic RVF, and it is safe and well tolerated.

Practical recommendations

Early diagnosis and treatment initiation of PAH and CTEPH treatment preserves from RVF. Initiating therapy at an early stage is a hope of enhancing survival rates, attaining the most effective therapeutic equilibrium, and preventing the progression of RVF.

Metabolic modulation with meldonium in the cardiomyocyte shows positive effects on mitochondrial bioenergetics and overall RV function. The favourable effects of meldonium targeting RV are considered safe for use in clinical practice without any significant adverse events mentioned so far. Meldonium in the combination with the treatment for the primary disease improves the QoL and functional capacity in patient with chronic RVF.

The QoL questionnaire serves as a valuable tool to evaluate the effectiveness of RVF therapy, particularly showing improvements in physical domains. Nevertheless, it is important to validate these subjective findings with objective clinical changes.

List of publications on the topic of the Thesis

Publications

1. **Kigitovica, D.,** Rusa, E., Rudzitis, A., Skride, A. 2022. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia in 2021 according to the new definition. *Eur J Intern Med*. 106:152-153. Doi:10.1016/j.ejim.2022.08.030

2. Vilskersts, R., **Kigitovica, D.**, Korzh, S. et al. 2021. Protective Effects of Meldonium in Experimental Models of Cardiovascular Complications with a Potential Application in COVID-19. *Int J Mol Sci*. 23(1):45. Published 2021 Dec 21. Doi:10.3390/ijms23010045

3. **Kigitovica, D.**, Dzirnieks, K., Lejnieks, A., Dambrova, M., Skride, A., Vilskersts, R. Meldonium improves functional capacity in patients with right heart failure. *Submitted*.

4. Kauliņš, R., Rudzītis, A., Lejnieks, A., **Kigitoviča, D.**, Skride, A. 2023. Baseline Clinical Characteristics and Incidence of Chronic Thromboembolic Pulmonary Hypertension Patients in Latvia, 2019–2020. *Medicina (Kaunas).* 59(8):1426. Published 2023 Aug 6. doi:10.3390/medicina59081426

5. **Kigitovica, D.**, Sablinskis, M., Sablinskis, K., Rudzitis, A., Skride, A. 2019. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia in 2018. *Eur J Intern Med.* 65:e9-e10. doi:10.1016/j.ejim.2019.04.022

Reports and theses at international congresses and conferences

1. **Kigitovica, D.**, Korzh, S., Videja, M., Vilks, K., Makrecka-Kuka, M., Liepins, E., Skride, A., Dambrova, M., Vilskersts, R. *Meldonium Therapy Improves the Right Ventricle Function.* 4th Baltic Pulmonary Hypertension Conference, Riga, Latvia: 1st Place Award Certificate.

2. **Kigitovica, D.**, Dzirnieks, K., Sablinskis, M., Lejnieks, A., Vilskersts, R. & Skride, A. *Meldonium increases functional capacity of patients with chronic right ventricular failure.* In: Medicina (Kaunas). 59, 2, 212. RSU Research week 2023: Knowledge for Use in Practice, Riga, Latvia.

3. **Kigitoviča, D.**, Korzh, S., Vidējā, M., Vilks, K., Makrecka-Kuka, M., Liepiņš, E., Skride, A., Dambrova, M. & Vilšķērsts, R. *Meldonium improves functioning of the right ventricle and mitochondrial bioenergetics in experimental model of pulmonary hypertension.* In: 24 Mar 2021, 139. RSU Research week 2021: Knowledge for Use in Practice, Riga, Latvia.

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Scientific awards

1. L'Oréal Baltic, in cooperation with the Baltic Academies of Sciences and the UNESCO National Commissions, Award for Women in Science, 2021.

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