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THE INFLUENCE OF SUBCHRONIC CO-APPLICATION OF VITAMINS B6 AND FOLIC ACID ON CARDIAC OXIDATIVE STRESS AND BIOCHEMICAL MARKERS IN MONOCROTALINE-INDUCED HEART FAILURE IN MALE WISTAR ALBINO RATS

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ABSTRACT

The aim of this study was to test the hypothesis that subchronic co-application of vitamins B6 and folic acid (FA) could affect heart failure (HF) induced by monocrotaline (MCT), with the modulation of oxidative stress parameters and cardiometabolic biomarkers. Biochemical and histomorphometric analysis were assessed in blank solution-exposed controls (C1 physiological saline 1ml/kg one day n=8; C2 physiological saline 1ml/kg 28 days n=8), MCT-induced HF (MCT 50mg/kg, n=8), B6+FA (vitamin B6 7mg/kg/day, FA 5mg/kg/day; n=8), and MCT+B6+FA (MCT 50mg/kg, vitamin B6 7mg/kg/day, FA 5mg/kg/day; n=8) in male Wistar albino rats (b.w. 160 g, at start). Superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, thiol-, carbonyl groups, and nitrotyrosine were determined in cardiac tissue. Echocardiography was performed in order to confirm MCT-induced HF. The right ventricular wall hypertrophy, accompanied with significant increase of troponin T and preserved renal and liver function has been shown in MCT-induced HF. However, these effects were not related to antioxidant effects of vitamin B6 and FA, since several parameters of oxidative stress were more pronounced after treatment. In this study co-application of vitamins B6 and folic acid did not attenuate hypertrophy of right ventricle wall, but aggravated oxidative stress which is involved in HF pathogenesis.

Keywords: heart failure, monocrotaline, vitamin B6, folic acid, oxidative stress, rat

1. Introduction

One of the experimental methods for inducing heart failure (via pulmonary arterial hypertension-PAH) is achieved by the application of monocrotaline (MCT). Pulmonary arterial hypertension (PAH) is characterized by remodeling mechanisms of the pulmonary arterial vessels with increased pulmonary vascular resistance and right ventricular afterload as a consequent, which leads to the development of right ventricular hypertrophy, right-sided heart failure, decompensation and ultimately to fatal outcome (Bogaard et al. 2009; Tuder RM et al. 2009; McLaughlin 2011). MCT is an 11-membered macrocyclic pyrrolizidine alkaloid derived from the seed of the *Crotalaria spectabilis* plant. Alkaloid monocrotaline is converted into reactive pyrrole metabolite dehydromonocrotaline in the liver, in a reaction catalyzed by cytochrome P450. It is deleterious for pulmonary endothelium and leads to imbalance between vasodilator and vasoconstrictor substances, causing changes in the vascular wall, increase in pulmonary resistance, right heart hypertrophy, and oxidative stress (Gomez-Arroyo et al. 2012).

Reactive oxygen species (ROS) are involved in the development of pathological ventricular hypertrophy. The oxidative stress may be responsible of proapoptotic pathways found in the failing RV in PAH (Buermans et al. 2005) but may also account for other aspects of pathological remodeling.

Lately, a lot of attention has been paid to B complex vitamins and their effects on cardiovascular system. Vitamin B6 is a hydrosoluble cofactor, which exerts antioxidant and anti-inflammatory properties (Stocker et al. 2003; Chen and Xiong 2005; Matxain et al. 2006; Friso et al. 2004). It has beneficial effect on cardiovascular system by reducing inflammation and degree of oxidative stress (Martinez et al. 2000). Low vitamin B6 levels may be a risk

factor for coronary disease due to increase in homocysteine levels (Friso et al. 2004; Martinez et al. 2000). Deficiency of vitamin B6 increases lipid peroxidation and reduces antioxidant defenses, and therefore can be associated with atherogenesis (Matxain et al. 2009; Keles et al. 2010).

Folic acid – vitamin B9 (FA) is essential for nitric oxide (NO) synthesis (Verhaar 1998) and therefore is necessary for proper functioning of endothelium (Chambers 2000). Various studies have shown that FA reduces plasma homocysteine levels (Joseph and Loscalzo 2013). Moens et al. (Moens et al. 2008) have observed that the administration of folate reduced myocardial ischemia and post reperfusion injury in rats. In a study on the isolated rat heart, it was found that perfusion of the heart with folic acid significantly increased the flow through the coronary arteries and reduced formation of superoxide anion, but led to the increase of index of lipid peroxidation (Djuric et al. 2007).

However, despite the results published in various studies, there is still no specific data on the association of B complex vitamins with chronic heart failure. Thus, the aim of this study was to test the hypothesis that combined application of vitamin B6 and FA could affect heart failure by modulating oxidative stress parameters and metabolic biomarkers.

2. Materials and Methods

Animal ethics statement

Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and European Directive for welfare of laboratory animals No: 2010/63/EU. The study has been approved by the Ethical Council for the Welfare of Experimental Animals, Ministry of Agriculture, Forestry and Water Management, Veterinary Directorate, Republic of Serbia (Number: 323-07-01339/2017-05/2).

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2.1. Experimental Animals

Male *Wistar albino* rats, with a body weight approximately 160 g and age of 25 - 30 days at the beginning of the experiment, were used in the experiments. The rats were housed in pairs in transparent Plexiglas cages with a wood-chip floor. Food and water were available *ad libitum*, and the ambient conditions were constant (temperature 21 ± 2 °C; humidity 55 ± 5 %; 12 h light-dark cycle with the light period beginning at 07:30 a.m.).

2.2. Experimental Protocol

The experiments were conducted during a 4-week period. The animals were divided into 5 groups: 1) C1- control (physiological saline 1ml/kg i.p. one day; n=8); 2) C2- control (physiological saline 1ml/kg i.p. 28 days; n=8); 3) MCT- monocrotaline group (MCT 50mg/kg in physiological saline 1ml/kg i.p. one day; n=8,); 4) B6+FA (vitamin B6 7mg/kg in physiological saline plus FA 5mg/kg in physiological saline 1ml/kg i.p. for 28 days; n=8); 5) MCT+B6+FA (MCT 50mg/kg in physiological saline 1ml/kg i.p. one day and vitamin B6 7mg/kg in physiological saline plus FA 5mg/kg in physiological saline 1ml/kg i.p. for 28 days; n=8). Heart failure was induced with a single intraperitoneal (i.p.) administration of monocrotaline at a dose of 50 mg/kg body weight, and confirmed by echocardiography between C1 and MCT groups. After 28 days animals were sacrificed. Heart tissue and blood samples of all animals were collected for histomorphometric and biochemical analysis.

2.3. Echocardiographic Assessment of Heart Morphology and Function

Cardiac volume and function were estimated in conscious tranquilized rats (acepromazine 0.5 mg/kg i.m. 15 min before examination) using a commercial echocardiograph ALOKA ProSound 2 (with 13 MHz linear probe). B- and M-modes were obtained in right parasternal

short axis view at the papillary muscles' level. Interventricular septum in diastole (IVSd), left ventricular diastolic internal diameter (LVIDd), posterior wall thickness at diastole (PWd), interventricular septum in systole (IVSs), left ventricular systolic internal diameter (LVIDs), posterior wall thickness at systole (PWs) and right ventricular internal diameter (RVID) were measured in M-mode. Other parameters were calculated according to Teicholz's formulas (Brown et al. 2002): left ventricular fractional shortening FS (%) = $\frac{(LVIDd - LVIDs)}{LVIDd} \times 100$; left ventricular end diastolic volume EDV (mL) = $1.047 \times (LVIDd)^3$; end systolic volume LVESV (mL) $1.047 \times (LVIDs)^3$; stroke volume, SV (mL) = EDV–ESV and left ventricular ejection fraction LVEF (%) = $\frac{SV}{EDV} \times 100$.

2.4. Histomorphometric Analysis

Heart tissue was properly oriented and cut transversely in 3 mm sections, placed in 4% neutral buffered formaldehyde and fixed for 24 hours by the immersion procedure. Afterwards it was embedded in paraffin, cut in 5µm serial sections until the complete heart wall thickness appeared, and stained with hematoxylin and eosin. For gross morphology, multiple measurements (10 per heart) were made from the endocardium to epicardium of the left ventricle free wall (LV wall thickness), right ventricle (RV wall thickness) and interventricular septum (IVS thickness). Average dimension of cardiomyocyte was calculated by measuring the diameter of 100 cardiomyocytes (at the nucleus level) of the LV free wall. All slides were analyzed using an Olympus BX 41 microscope and photographed with Olympus C–5060 wide zoom digital camera and Olympus DP-soft Image Analyzer program. All values are expressed in micrometers (µm).

2.5 Biochemical Parameters Determination

Serum homocysteine (Hcy) was measured by an automated electrochemiluminescence immunoassay system ADVIA Centaur XP system (Siemens Healthcare GmbH, Erlangen, Germany). The serum troponin T (hsTnT), glucose level, urea (UREA), creatinine (CREA), uric acid (UA), the lipid profile parameters (total cholesterol – CHL, low density lipoprotein – LDL, high density lipoprotein – HDL, and triglycerides – TGL), total proteins (TP), albumin (ALB), aspartate-aminotransferase (AST), alanine - aminotransferase (ALT), α -amylase (α -AMY), alkaline phosphatase (ALP), C- reactive protein (CRP) and interleukin 6 (IL 6) were analyzed using spectrophotometry and commercial kits (Siemens Healthcare Diagnostics Ltd. Frimley, Camberley, UK) on an automatic biochemical analyzer (Dimension Xpand, Siemens). For determination of haemostatic factors (fibrinogen and vonWillebrand Factor (vWF) activity) immunoturbidimetric commercial assay (Siemens Healthcare GmbH, Marburg, Germany) was used.

2.6. Preparation of the Heart Tissue Samples

After isolation, the heart was washed with saline (0.9% NaCl) and dried on filter paper. It was homogenized in 50 mM RIPA buffer (Radio Immunoprecipitation Assay) pH 7.4 with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, Missouri, USA), and then centrifuged at 14,000 rpm for 30 minutes. Cardiac tissue was frozen at -80°C until the time of analysis.

2.7. Determination of Oxidative Stress Parameters in Cardiac Tissue

Oxidative stress parameters were measured in cardiac tissue homogenate. Superoxide dismutase (SOD) activity was determined spectrophotometrically (Misra and Fridovich 1972.). Glutathione peroxidase (GPX) activity was determined by the coupled assay procedure (Gunzler et al. 1974). One unit of enzyme activity is reported as mmol NADPH

(Nicotinamide adenine dinucleotide phosphate) oxidized per minute, assuming 6.22×10^3 /L/mol/cm to be the molar absorbance of NADPH at 340 nm. The amount of thiol (P-SH) groups was determined according to the method of Jocelyn (1987) and expressed as mmol/g of proteins. Total glutathione (GSH) was determined spectrophotometrically and expressed as nmol/mg of proteins (Ravindranath 1994). Reactive carbonyl derivatives (RCD) were determined using the method of Levine et al. (1994). The protein oxidation level was monitored by determination of a classic carbonyl reagent, 2, 4-dinitrophenylhydrazine (DNPH) activity of proteins. Spectrophotometric measurement of RCD was performed and expressed as mmol/g proteins. An ELISA test was used to measure nitrotyrosine content in rat heart tissue. Nitrotyrosine content was determined based on standard curves (Peluffo and Radi 2007), and expressed as nmol/L. Protein concentration was determined using Bicinchoninic Acid Protein Assay kit (BCA-1, Sigma- Aldrich, St. Louis, Missouri, USA).

2.8. Statistical analysis

Continuous variables with normal distribution were expressed as means $\pm$ standard deviations. Discrete variables were presented as frequencies and percentages. Analysis of variance (ANOVA) with *post hoc* analysis, as well as a non-parametric variant of the Kruskal-Wallis test was used to compare the average values of the parametric characteristics between the groups, depending on the data distribution. The value of $p < 0.05$ was considered statistically significant.

3. Results

Based on echocardiographic assessment of heart morphology and function, histomorphometric and biochemical analysis, we confirmed monocrotaline induced heart failure model. The influence of co-application of vitamin B6 and folic acid on

histomorphometric, biochemical and oxidative stress parameters in this model was also determined.

3.1. Echocardiographic Assessment of Heart Morphology and Function

Overt signs of monocrotaline-induced heart failure emerged merely two week period: Right ventricular internal diameter (RVID) was statistically significantly increased in comparison to baseline monocrotaline-treated rats ($0,21 \pm 0,04$ cm vs. $0,11 \pm 0,01$ cm and $0,28 \pm 0,04$ cm vs. $0,11 \pm 0,01$ cm in third and fourth week, respectively) and in comparison to control group ($0,21 \pm 0,04$ cm vs. $0,11 \pm 0,01$ cm and $0,28 \pm 0,04$ cm vs $0,11 \pm 0,01$ cm in third and fourth week, respectively). There were no changes in left ventricular ejection fraction (EF) in comparison to baseline monocrotaline-treated rats ($85.53 \pm 1.42\%$ vs. $86.56 \pm 1.27\%$ and $82.72 \pm 1.43\%$ vs. $86.56 \pm 1.27\%$ in third and fourth week, respectively) and control group were observed ($85.53 \pm 1.42\%$ vs. $86.25 \pm 1.70\%$ and $82.72 \pm 1.43\%$ vs. $87.76 \pm 1.60\%$ in third and fourth week, respectively) (Table 1). Figure 1 shows a typical echocardiogram of rat's heart morphology changes: before treatment, 7 days, 14 days, 21 days and 28 days post treatment.

3.2 Histomorphometric analysis

Right ventricle wall thickness was significantly thicker in MCT group compared to C1 group (1229.63 ± 231.80 μ m vs. 731.55 ± 131.26 μ m). There was no statistically significant difference in wall thickness of left ventricle and interventricular septum but the tendency of reducing both of these parameters between MCT and C1 group was noticed. Average

dimension of cardiomyocyte was statistically significant higher in MCT group in comparison with C1 group ($21.20 \pm 1.55 \mu\text{m}$ vs. $17.99 \pm 1.90 \mu\text{m}$).

There were no differences between B6+FA and C2 group in all analyzed histomorphometric parameters, but RV wall thickness was significantly higher in MCT+B6+FA group compared to B6+FA group ($1346.06 \pm 304.01 \mu\text{m}$ vs. $914.70 \pm 134.42 \mu\text{m}$).

LV free wall thickness was significantly higher in MCT+B6+FA group compared to MCT group ($2453.48 \pm 237.89 \mu\text{m}$ vs. $1676.26 \pm 208.07 \mu\text{m}$). There was a trend towards thicker IVS in MCT+B6+FA group compared to MCT group ($2163.97 \pm 580.50 \mu\text{m}$ vs. $1651.02 \pm 293.95 \mu\text{m}$) and C2 group ($2163.97 \pm 580.50 \mu\text{m}$ vs. $2112.61 \pm 296.05 \mu\text{m}$) but it did not reach statistical significance. Average dimension of cardiomyocyte of the LV free wall was not significantly different between MCT+B6+FA and MCT group ($21.73 \pm 2.50 \mu\text{m}$ vs. $21.20 \pm 1.55 \mu\text{m}$), but there were significant difference between MCT and C group ($21.20 \pm 1.55 \mu\text{m}$ vs. $17.99 \pm 1.90 \mu\text{m}$). Results are presented in Table 2 and Figure 2.

3.3 Biochemical analysis

Administration of MCT resulted in significant increase of troponin T values compared to C1 group [$199 (58-251) \text{ pg/ml}$ vs. $17 (16-25) \text{ pg/ml}$]. Serum Hcy levels, as well as markers of renal and liver function were not different between these two groups (UREA, CREA, AST, ALT, and ALP respectively). Mean glucose levels were significantly higher in MCT group in comparison with C1 group ($7 \pm 1.20 \text{ mmol/L}$ vs. $6 \pm 0.63 \text{ mmol/L}$). Serum HDL value was significantly decreased in MCT group in comparison with C1 group ($0.84 \pm 0.26 \text{ mmol/L}$ vs. $1.21 \pm 0.13 \text{ mmol/L}$), but there were no differences in other lipid profile parameters, CHL and TGL, between these two tested groups, respectively ($1.37 \pm 0.24 \text{ mmol/L}$ vs. $1.38 \pm 0.15 \text{ mmol/L}$ and $0.95 \pm 0.33 \text{ mmol/L}$ vs. $0.89 \pm 0.16 \text{ mmol/L}$). Albumin level and total protein

content were significantly lower between MCT and C1 group (391.55 ± 156.74 g/L vs. 497.90 ± 71.15 g/L and 52.55 ± 5.39 g/L vs. 62.20 ± 2.35 g/L, respectively). The content of uric acid in C1 group was not different in comparison to MCT group (85.45 ± 22.96 μ mol/L vs. 74.40 ± 14.28 μ mol/L). Mean α -AMY levels were significantly lower in MCT group compared to C1 group (2530.00 ± 657.01 U/L vs. 3560.75 ± 580.49 U/L). In all tested groups CRP in each sample was under 2 mg/L (limit detection). In 100% of samples in all tested groups, CRP was under 2 mg/L (limit detection). Almost the same situation was with IL 6 (100% of samples were under 1.5 mg/L in all evaluated groups). The values of fibrinogen were under 1.8 g/L in all samples in C1 and C2, group and in 75% of samples in MCT group, but von Willebrand factor (vWF) value was significantly higher in MCT group in comparison with C1 group (227.65 ± 41.01 IU vs. 156.00 ± 37.19 IU) (Table 3).

Troponin T (hsTnT) remained elevated after co-application of vitamin B6 and folic acid, though with a lower extent (163 (119-369) nmol/L vs. 199 (58-251) nmol/L). There was no difference in Hcy between the groups, even in MCT+B6+FA group. Cholesterol (CHL) and triglycerides (TGL) were significantly higher in MCT+B6+FA group in comparison with MCT (2.46 ± 1.32 mmol/L vs. 1.37 ± 0.24 mmol/L, 1.61 ± 1.15 mmol/L vs. 0.95 ± 0.33 mmol/L, respectively) and B6+FA groups (2.46 ± 1.32 mmol/L vs. 1.67 ± 0.23 mmol/L, 1.61 ± 1.15 mmol/L vs. 0.91 ± 0.2 mmol/L, respectively). Levels of UA, UREA, CREA, as well as AST and ALT were not different between the tested groups. The content of ALP was significantly lower in MCT+B6+FA in comparison with MCT and B6+FA groups. Mean α -AMY levels were significantly higher in MCT+B6+FA group compared with MCT and B6+FA group. The value of TP significantly decreased in MCT+B6+FA group compared with B6+FA group. The values of CRP and IL 6 were under 2 mg/L and 1.5 mg/L, respectively, in all samples in MCT+B6+FA group, as well as in all tested groups. The content of fibrinogen was in 50% of samples in MCT+B6+FA group under 1.8 g/L, and von Willebrand factor

(vWF) value significantly decreased in MCT+B6+FA group in comparison with MCT group (141.84 ± 115.84 IU vs. 227.65 ± 41.01 IU) (Table 3).

3.4. Oxidative stress parameters

Antioxidant defense system, including key antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX) and glutathione, together with parameters of oxidative damage of proteins, thiol- and carbonyl groups were measured. Treatment with monocrotaline induced increase the activity of both antioxidant enzymes, SOD and GPX, which in case of GPX reached statistical significance (390.60 ± 68.46 U/mg of proteins vs. 540.75 ± 147.36 U/mg of proteins and 402.83 ± 50.92 U/mg of proteins vs. 1276.10 ± 172.34 U/mg of proteins, respectively) (Figure 3 and Figure 4). In contrast, the total glutathione content was significantly lower in MCT group compared to C1 group (58.83 ± 14.75 μ mol/g of proteins vs. 25.70 ± 3.08 μ mol/g of protein) (Figure 5). The level of thiol protein, group as sacrificial antioxidants, was also significantly reduced in MCT group (387.36 ± 73.92 μ mol/g of proteins vs. 202.64 ± 28.35 μ mol/g of protein) (Figure 6), while the content of carbonyl groups was decreased in MCT group in comparison with C1 group (25.84 ± 6.79 μ mol/g of proteins vs. 42.06 ± 10.29 μ mol/g of proteins) (Figure 7).

Following vitamin B6 and folic acid co-application, activity of SOD was significantly decreased in comparison to MCT and B6+FA group (372 ± 37.07 U/mg of protein vs. 540.75 ± 147.36 U/mg of protein, 372 ± 37.07 U/mg of protein vs. 486.60 ± 64.147 U/mg of protein, respectively) (Figure 3). GPX activity showed similar trend in comparison to MCT group but without reaching statistical significance, while in comparison with B6+FA group the values were significantly higher (1094.21 ± 285.08 U/mg of protein vs. 1276.09 ± 172.34 U/mg of protein, 1094.21 ± 285.08 U/mg of protein vs. 226.53 ± 56.02 U/mg of protein, respectively)

(Figure 4). The values of total glutathione after vitamins B6 and FA co-administration were statistically significant decreased in comparison to MCT group (15 ± 4.64 mmol/mg of proteins vs. 25.70 ± 3.08 mmol/mg of proteins), and also there was statistically significant decrease in B6+FA group in comparison with C2 group (30.76 ± 5.98 mmol/mg of proteins vs. 70.62 ± 11.44 mmol/mg of proteins) (Figure 5). The co-application of vitamins B6 and folic acid induces further decrease of the content of thiol groups, but not high statistically significant difference, in comparison to both MCT and C1 group, but there was statistically significant decrease in B6+FA group in comparison with C2 group (143.43 ± 49.82 U/mg of protein vs. 202.64 ± 28.35 U/mg of protein, 143.43 ± 49.82 U/mg of protein vs. 387.35 ± 73.92 U/mg of protein, 137.77 ± 63.84 U/mg of protein vs. 308.45 ± 39.24 U/mg of protein, respectively) (Figure 6). The reactive carbonyl derivatives showed similar trend in comparison to MCT group but without reaching statistical significance (16.32 ± 6.32 μ mol/g of proteins vs. 25.84 ± 6.79 μ mol/g of proteins) (Figure 7). As well as thiol and carbonyl groups, content of nitrotyrosine was also reduced in MCT group in comparison with C1, but there was not statistically significant difference (7 ± 2 nmol/L vs 9 ± 3 nmol/L) (Figure 8).

4. Discussion

The most commonly used animal models of PH and consequent right heart failure are the chronic hypoxic model and the monocrotaline (MCT) injury model. The MCT rat model is favored by many investigators, because of a very simple and thus technically appealing animal model. The monocrotaline pyrrole (MCTP) has to be activated to the reactive bifunctional cross-linking compound MCTP in the liver, which leads to vascular injury. The response to monocrotaline is variable among species, strains, and even animals which is caused by differences in the cytochrome P-450 hepatic metabolism (Huxtable 1990; Campian

et al. 2006). The exact mechanism of MCT-induced PH is not known, but it is speculated by many investigators that it causes direct pulmonary endothelial damage which leads to an increase pulmonary vascular resistance (PVR) and the right ventricle compensates by hypertrophy and eventually fails. We used echocardiographic assessment for approving this MCT animal model. Therefore, because the right ventricle was affected, in this short time in which we followed them, it was not possible to significantly develop any influence of the right ventricle on the reduction of the left lumen, i.e. on changing parameters related to the left ventricle.

Nutrients such as folic acid, vitamins B6, B12 and C, L-arginine, albumin and magnesium have a role in HF. Co-application of these nutrients in specific combinations make significant benefit such as the possibility that they are unlikely to produce significant side effects (because they are natural and endogenous with the body). The exact correlations between the plasma levels of folic acid, vitamins B6, B12 and C, L-arginine, albumin, magnesium and the severity of HF remain to be investigated, as well as to what extent these diseases could be reversed by these nutritional factors. Vitamin B6 reduces inflammation and degree of oxidative stress in cardiovascular system. Deficiency of vitamin B6 increases lipid peroxidation and reduces antioxidant defenses, and therefore can be associated with atherogenesis. Folic acid - B9 (FA) is necessary for proper functioning of endothelium (Chambers 2000).

Although vitamin B6 and folic acid have antioxidant and anti-inflammatory effects (Lotto et al. 2011; Joshi et al. 2001) and are used in primary prevention of coronary heart disease, we found significant thickening of the LV wall diameter and the same tendency in IVS diameter in MCT+B6+FA groups. It might be due to direct influence of these substances in heart or secondary to cardiac remodeling.

Most of the studies with vitamin B6 and folic acid are done in healthy population, without pre-existing cardiovascular disease. It is well known that higher B-vitamins and folate intake is beneficial and is associated with the lower coronary heart disease risk (Alfthan et al. 2003; Tucker et al. 1996; Medrano et al. 2000). On the other hand, several studies did not found the decrease in coronary heart disease risk (Bazzano et al. 2006; Clarke et al. 2010; Li et al. 2016; Miller et al. 2010). Other study done in women indicate that long-term, combined treatment with folic acid, vitamin B6, and vitamin B12 does not alter biomarkers of inflammation and endothelial dysfunction (Christen et al. 2018). It was also suggested that B-vitamins intake may have protective effects in primary prevention but not in secondary (Jayedi and Zargar 2018). Many data from vitamin supplementation studies demonstrated that vitamin B6 is not effective for preventing the recurrence of cardiovascular events (Wilmink et al. 2004; Albert et al. 2008; Toole et al. 2004). Vitamin B6 and folic acid supplementation is considered to have two sides, where a beneficial effect may be observed with nutritional support in the prevention phase, and a disease-aggravating effect may be observed after the onset of illness. It is assumed that vitamin B supplementation is effective in patients without a cardiovascular event, while ineffective in patients who have already had it (Lotto et al. 2011). In our study vitamin B6 and folic acid were administered after induction of heart failure, corroborating the fact that they might have harmful effects in already damaged heart.

Our study has shown that rats in monocrotaline-induced model of heart failure exhibit oxidative distress based on decreased levels of protein thiol groups, total glutathione and RCD content, which were accompanied by increase in activities of SOD and GPX. Oxidative distress represents a pathologic condition in which the balance of oxidant generation and detoxification is tipped toward a prooxidant state, antioxidant defenses are overwhelmed and reactive species accumulate. In the pathophysiology of heart failure, it has been suggested that oxidative distress can be a consequence of increased circulating neurohormones contra

balance, and hemodynamic disorder, together with inflammation and decreased oxygen delivery. On the other hand, disturbed redox balance in heart failure might contribute to further impairment of cardiac function, either by oxidative damage to vital cellular molecules, or by affecting cell signaling involved in apoptosis 3. The negative side of the ROS is reflected through the activation of a wide range of signaling pathways related to cell death, proliferation of cardiac fibroblasts, activation of matrix metalloproteinases, mtDNA damage, mitochondrial dysfunction, impaired calcium handling and contractility, and cardiac hypertrophy, all of which ultimately leading to maladaptive myocardial remodeling and cardiac dysfunction. SOD and GPX act in a mutually supportive fashion to remove excess of superoxide, H_2O_2 and other lipid peroxides. SOD converts superoxide anions to H_2O_2 , which are further reduced to water molecules by the activity of GPX.

After the combined treatment with B6 and folic acid we observed the detrimental effect of this co-application regarding oxidative stress. Namely, thiol group content were 2-times lower than in MCT group, while total glutathione was also decreased. The nitrotyrosine content modified with the same trend. The formation of nitrotyrosine represents a specific peroxynitrite-mediated protein modification and is considered as a biomarker for endogenous peroxynitrite activity. Our results correlated with results of Baez et al. (2018). It is tempting to speculate that contributing factor to enhance oxidative stress after co-application was decreased activity of SOD. Specifically, adaptive increase in SOD activity which was observed after MCT treatment was abolished by vitamin B6 and FA administration, while GPX remained the same. Recent literature data suggest that vitamin B6 has capacity to neutralize superoxide anion, which might reverse adaptive upregulation of SOD, further potentiating profound oxidative modifications of key macromolecules (Mahfouz et al. 2009). In the view of the findings that after the onset of disease, vitamin B6 and FA have disease-aggravating effect these results are biologically plausible, although the mechanisms

responsible remain unknown. Similar decreases in SOD activity was observed in pregnant-women after vitamins supplementation (Hunaiti and Saleh 1996).

Antioxidant enzyme activities in MCT-induced heart failure seem to differ with respect to the cellular type and compartment, and the results of various studies have been conflicting. Thus, Mohammadi et al. (2012) found that levels of extracellular SOD and GSH-Px in MCT treated rats were significantly decreased four weeks after treatment. Similar down-regulation in antioxidant enzymes in MCT- induced heart failure was also found in Soleus (SOL-oxidative and slow twitching muscle) muscle tissue (Bertaglia et al. 2011).

On the other hand, the investigation of Pichardo et al. (1999), who measured the activities of antioxidant enzymes 3 weeks after MCT application, showed no difference between MCT group and controls. Moreover, Farahmand et al. (2004), in accordance with our results, showed significant increase in antioxidant enzymes activity in cardiac tissue, accompanied with the increase in septal wall thickness 2 weeks after MCT application. Cardiac muscle is not the only muscle tissue initially responding by upregulation of these enzymes after MCT treatment. Thus, in a rat model of pulmonary hypertension induced by 60 mg/kg MCT, the protein expression of SOD2 and catalase were significantly increased in the diaphragm muscles from the MCT treated rats. Taken together these data suggest complex transcriptional regulation of antioxidant enzymes after MCT treatment that needs to be further explored. Tissue specific expression of antioxidant enzymes isoforms further contributes to the heterogeneity in response towards MCT. In this line, it should be mentioned that stage specific pathobiological changes have been shown to play role in the course of left ventricular failure development, where a biphasic response to oxidative stress was proposed. Namely, in the stage of left ventricular hypertrophy a significant increase in antioxidant enzyme activities, due to their reactive upregulation in response to oxidative stress, was observed, while the failure stage was characterized by a fall in the antioxidant enzyme activities and

consequent increase in oxidative stress biomarkers. Interestingly, in the aforementioned study of Farhmand et al. (2004), initial upregulation of antioxidant enzyme activities after MCT administration was followed by their decrease and enhanced lipid peroxidation 6 weeks after treatment, suggesting that similar biphasic pattern of changes may also exist in the development of right ventricular failure, at least in a MCT model.

Many studies have been shown that the RV is more susceptible to the oxidative stress than the LV in the setting of heart failure because of the RV weakness in antioxidant-adaptive defense mechanisms (Reddy and Bernstein 2015; Schreckenberget al. 2015; Borchiet al. 2010). Schreckenberget al. (2015) found that the RV is exposed to higher oxidative stress damage as detected by dihydroethidium staining for superoxide and measurements of peroxynitrite. The quantification of nitrotyrosine indirectly demonstrates the production of peroxynitrite and oxidative damage in endothelial cells (Baez et al. 2018). There were differential superoxide levels between the two ventricles which may be caused by varied expression of manganese superoxide dismutase. That study showed that in condition of nitric oxide deficiency RV lacks the ability to upregulate manganese superoxide dismutase, and also that specific protein oxidation can occur in SU5416/ovalbumin-induced pulmonary hypertension model of RV failure. Total protein carbonylation was not altered in the RVs of rats with pulmonary hypertension compared with the controls (Wang et al. 2017), also observed in our study.

5. Conclusion

In conclusion, MCT led to right heart failure, but levels of liver and kidney markers were not elevated that indicates neither hepatotoxicity nor nephrotoxicity. Also, there was not recognized inflammation in treated animals. In this study we observed that co-application of vitamin B6 and folic acid did not attenuate hypertrophy of RV wall, but aggravated oxidative stress which is involved in pathogenesis of PH and subsequent HF.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgement

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Conflicts of Interest

The authors have no conflicts of interest to declare

References

Albert CM, Cook NR, Gaziano JM, et al. 2008. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA*. 299: 2027–2036

Alfthan G, Laurinen MS, Valsta LM, Pastinen T, Aro A. 2003. Folate intake, plasma folate and homocysteine status in a random Finnish population. *European Journal of Clinical Nutrition*. 57 (1): 81–8

Baez MC, Scribano MP, Tarán M. 2018. Nitrotyrosine as a biomarker: recovery of histopathological atherogenic lesions in rats treated with ascorbic acid. *Trends Med*. 18(4):5-5

Bazzano LA, Reynolds K, Holder KN, He J. 2006. Effect of folic acid supplementation on risk of cardiovascular diseases: A meta-analysis of randomized controlled trials. *Jama*. 296 (22): 2720–6

Bertaglia RS¹, Reissler J, Lopes FS, Cavalcante WL, Carani FR, Padovani CR, et al. 2011. Differential morphofunctional characteristics and gene expression in fast and slow muscle of rats with monocrotaline-induced heart failure. 42(3):205-15

Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. 2009. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest* 135: 794 –804

Borchi E, Bargelli V, Stillitano F, Giordano C, Sebastiani M, Nassi PA, d'Amati G, Cerbai E, Nediani C. 2010. Enhanced ROS production by NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. *Biochim. Biophys. Acta* 1802: 331–338.

Brown, Lindsay, Andrew Fenning, Vincent Chan, David Loch, Kathleen Wilson, Bonita Anderson, Darryl Burstow. 2002. Echocardiographic Assessment of Cardiac Structure and Function in Rats. *Heart, Lung & Circulation* 11 (3): 167–73.

Buermans HP, Redout EM, Schiel AE, Musters RJ, Zuidwijk M, Eijk PP, van Hardeveld C, Kasanmoentalib S, Visser FC, Ylstra B, Simonides WS. 2005. Microarray analysis reveals pivotal divergent mRNA expression profiles early in the development of either compensated ventricular hypertrophy or heart failure. *Physiol Genomics* 21: 314–323

Campian ME, Hardziyenka M, Michel MC, et al. 2006. How valid are animal models to evaluate treatments for pulmonary hypertension? *Naunyn Schmiedeberg's Arch Pharmacol* 373: 391–400

Chambers JC. 2000. Improved vascular endothelial function after oral B vitamins: an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation*. 102: 2479–83

Chen H and Xiong L. 2005. Pyridoxine is required for post embryonic root development and tolerance to osmotic and oxidant stresses. *Plant. J.* 44: 396-60

Christen WG, Cook NR, Van Denburgh M, Zaharris E, Albert CM, Manson JE. 2018. Effect of Combined Treatment With Folic Acid, Vitamin B₆, and Vitamin B₁₂ on Plasma Biomarkers of Inflammation and Endothelial Dysfunction in Women. *J Am Heart Assoc.* 18; 7(11).

Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, Bonna KH, Spence JD, Nygard O, Jamison R, et al. 2010. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. *Archives of Internal Medicine.* 170 (18): 1622

Djuric D, Vusanovic A, Jakovljevic V. 2007. The effects of folic acid and nitric oxide synthase inhibition on coronary flow and oxidative stress markers in isolated rat heart. *Mol.Cell.Biochem.* 300(1-2): 177-83

Farahmand F, Hill MF, Singal PK. 2004. Antioxidant and oxidative stress changes in experimental cor pulmonale. 260(1-2):21-9

Friso S, Girelli D, Martinelli N, Olivieri O, Lotto V, Bozzini C, et al. 2004. Low plasma vitamin B6 concentrations and modulation of coronary artery disease risk. *Am. J. Clin. Nutr.* 79:992-8

Gomez-Arroyo JG, Farkas L, Alhussaini AA, et al. 2012. The monocrotaline model of pulmonary hypertension in perspective. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 302: L363-9

Gunzler WA, Kremers H, Flohe L. 1974. An improved coupled test procedure for glutathione peroxidase in blood. *ZeitschriftFuerKlinischeChemie Und KlinischeBiochemie* 12:444-8

Hunaiti AA1, Saleh MS. 1996. Effects of iron, zinc, calcium, and vitamins on the activity and contents of human placental copper/zinc and manganese superoxide dismutases. *Biol Trace Elem Res.* 54(3):231-8.

Huxtable RJ. Activation and pulmonary toxicity of pyrrolizidine alkaloids. 1990. *Pharmacol Ther* 47: 371-389.

Jayedi A, Zargar MS. 2018. Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: a systematic review and dose-response meta-analysis of prospective cohort studies. *Crit Rev Food Sci Nutr.* 15: 1-11

Jocelyn PC. 1987. Spectrophotometric assay of thiols. *Methods Enzymol* 143:44- 67.

Joseph J, Loscalzo J. 2013. Methoxistasis: Integrating the roles of homocysteine and folic acid in cardiovascular pathobiology. *Nutrients*. 5(8): 3235-56

Joshi RS, Adhikari BS, Patro S, Chattopadhyay, Mukherjee T. 2001. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radical Biology and Medicine*. 30 (12): 1390–9

Keles M, Behcet A, Gumustekin K, Demircan B, Ozbey I, Akyuz M, et al. 2010. Antioxidative status and lipid peroxidation in kidney tissue of rats fed with vitamin B6-deficient diet. *Renal Failure*. 32(5): 618–622

Levine RL, Williams JA, Stadtman ER, Shacter E. 1994. Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol* 233:346–57.

Li Y, Huang T, Zheng Y, Muka T, Troup J, Hu FB. 2016. Folic acid supplementation and the risk of cardiovascular diseases: A meta-analysis of randomized controlled trials. *Journal of the American Heart Association*. 5(8):e003768

Lotto V, Choi SW, Friso S. 2011. Vitamin B6: a challenging link between nutrition and inflammation in CVD. *British Journal of Nutrition*. 106 (02): 183-195

Lowry OH, Rosebrough NJ, Farr AL, Randall TD. 1951. Protein measurement with the Folin phenol reagent. *J BiolChem* 193:265

Mahfouz MM1, Zhou SQ, Kummerow FA. 2009. Vitamin B6 compounds are capable of reducing the superoxide radical and lipid peroxide levels induced by H₂O₂ in vascular endothelial cells in culture. *Int J Vitam Nutr Res*. 79 (4):218-29

Martinez M, Cuskelly GJ, Williamson J, Toth JP, Gregory JF 3rd. 2000. Vitamin B-6 deficiency in rats reduces hepatic serine hydroxymethyltransferase and cystathionine beta-synthase activities and rates of in vivo protein turnover, homocysteine remethylation and transsulfuration. *J.Nutr*. 130: 1115-23

Martínez-Vega R, Garrido F, Partearroyo T, Cediel R, Zeisel SH, Martínez-Álvarez C et al. 2015. Folic acid deficiency induces premature hearing loss through mechanisms involving cochlear oxidative stress and impairment of homocysteine metabolism. *FASEB J.* 29 (2):418-32.

Matxain JM, Padro D, Ristila M, Strid A, Eriksson LA. 2009. Evidence of high OH radical quenching efficiency by vitamin B6. *J. Phys. Chem. B.* 113(29): 9629–9632

Matxain JM, Ristila M, Strid A, Eriksson LA. 2006. Theoretical study of the antioxidant properties of pyridoxine. *J. Phys. Chem. A.* 110(48): 13068–13072

McLaughlin VV. 2011. Looking to the future: a new decade of pulmonary arterial hypertension therapy. *Eur.Respir. Rev.* 20: 262–269

Medrano M J, Sierra MJ, Almazan J, Olalla MT, Lopez AG. 2000. The association of dietary folate, B6, and B12 with cardiovascular mortality in Spain: An ecological analysis. *American Journal of Public Health.* 90 (10): 1636–8

Miller ER, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. 2010. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. *American Journal of Cardiology.* 106 (4): 517–27

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* 247(10): 3170–3175

Moens AL, Champion HC, Claeys MJ, Tavazzi B, Kaminski PM, Wolin MS et al. 2008. High-dose folic acid pretreatment blunts cardiac dysfunction during ischaemia coupled to maintenance of high-energy phosphates and reduces postreperfusion injury. *Circulation.* 117:1810–1819

Mohammadi S1, Najafi M, Hamzeiy H, Maleki-Dizaji N, Pezeshkian M, Sadeghi-Bazargani H et al. 2012. Protective effects of methylsulfonylmethane on hemodynamics and oxidative stress in monocrotaline-induced pulmonary hypertensive rats. *Adv Pharmacol Sci.* 2012:507278

Peluffo G, Radi R. 2007. Biochemistry of protein tyrosine nitration in cardiovascular pathology. *Cardiovasc Res.* 75(2):291-302

Pichardo J, Palace V, Farahmand F, Singal PK. 1999. Myocardial oxidative stress changes during compensated right heart failure in rats. *Mol Cell Biochem.* 196(1-2):51-7

Ravindranath V. 1994. Animal models and molecular markers for cerebral ischemia-reperfusion injury in brain. *Methods Enzymol.* 233:610–619

Reddy S, Bernstein D. 2015. Molecular mechanisms of right ventricular failure. *Circulation* 132: 1734–1742.

Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. 2004. Trends in heart failure incidence and survival in a community-based population. *JAMA.* 292(3): 344-350

Schreckenber R, Rebelo M, Deten A, Weber M, Rohrbach S, Pipicz M, Csonka C, Ferdinandy P, Schulz R, Schlüter K.D. 2015. Specific Mechanisms Underlying Right Heart Failure: The Missing upregulation of superoxide dismutase-2 and its decisive role in antioxidative defense. *Antioxid. Redox Signal.* 23: 1220–1232.

Stocker P, Lesgards JF, Vidal N, Chaliier F, Prost M. 2003. ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochem Biophys Acta.* 162: 1-8

Toole JF, Malinow MR, Chambless LE et al. 2004. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the

Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA*. 291: 565–575

Tucker KL, Selhub J, Wilson PW, Rosenberg IH. 1996. Dietary intake pattern relates to plasma folate and homocysteine concentrations in the framingham heart study. *The Journal of Nutrition*. 126 (12): 3025–31

Tuder RM, Abman SH, Braun T, Capron F, Stevens T, Thistlethwaite PA, Haworth SG. 2009. Development and pathology of pulmonary hypertension. *J Am Coll Cardiol* 54: S3–S9

Verhaar MC. 1998. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation*. 97: 237–41

Wang X, Shults N.V, Suzuki Y.J. 2017. Oxidative profiling of the failing right heart in rats with pulmonary hypertension. *PLoS ONE*. 12(5):e0176887

Wilming AB, Welch AA, Quick CR, et al. 2004. Dietary folate and vitamin B6 are independent predictors of peripheral arterial occlusive disease. *J Vasc Surg*. 39: 513–516

Figure legends:

Figure 1: Echocardiograms of rats treated with saline (left panel) and rats treated with monocrotaline (right panel). Dash-lines denote right-ventricular internal diameter. Note severe dilatation of right ventricular diameter in third and fourth week post monocrotaline injection.

W1: first week; W2: second week; W3: third week; W4: fourth week

Figure 2: Representative histological slides of rat's hearts in experimental groups

Figure 3: Superoxide dismutase (SOD) activity in rat cardiac tissue

* – $P < 0.05$ versus C1 group, $\square\square$ – $P < 0.01$ versus MCT group, ^ – $P < 0.05$ versus B6+FA group

Figure 4: Glutathione peroxidase (GPX) activity in rat cardiac tissue

* * – $P < 0.01$ versus C1 group, ^ ^ – $P < 0.01$ versus B6+FA group

Figure 5: Glutathione content in rat cardiac tissue

* * – $P < 0.01$ versus C1 group, ### – $P < 0.01$ versus C2 group, ^ – $P < 0.05$ versus B6+FA group

Figure 6: The amount of thiol (P-SH) group in rat cardiac tissue

* * – $P < 0.01$ versus C1 group, # – $P < 0.05$ versus C2 group

Figure 7: The content of reactive carbonyl derivatives (RCD) in rat cardiac tissue

* * – $P < 0.01$ versus C1 group

Figure 8: Nitrotyrosine content in rat cardiac tissue

Table 1. Echocardiographic parameters affected by monocrotaline treatment

Parameter	Control					Monocrotaline				
	Baseline	W1	W2	W3	W4	Baseline	W1	W2	W3	W4
IVSd (cm)	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.17 ± 0.01*†	0.16 ± 0.01	0.16 ± 0.01
LVIDd (cm)	0.47 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.53 ± 0.01	0.44 ± 0.02	0.50 ± 0.02*	0.51 ± 0.02*	0.49 ± 0.02*	0.46 ± 0.01*†
PWd (cm)	0.23 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.27 ± 0.03	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.03
IVSs (cm)	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.01*	0.23 ± 0.01	0.22 ± 0.01
LVIDs (cm)	0.24 ± 0.02	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.21 ± 0.01	0.26 ± 0.01*	0.26 ± 0.02*	0.25 ± 0.02*	0.23 ± 0.01
PWs (cm)	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.36 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	0.32 ± 0.01†	0.32 ± 0.01†
EF (%)	86.13 ± 0.93	86.40 ± 1.31	86.26 ± 1.04	86.25 ± 1.70	87.76 ± 1.60	86.56 ± 1.27	84.60 ± 2.23	83.92 ± 3.78	85.53 ± 1.42	82.72 ± 1.43
FS (%)	48.12 ± 2.46	50.16 ± 1.55	53.08 ± 1.48	50.98 ± 2.23	52.82 ± 2.04	50.76 ± 1.78	49.21 ± 2.53	49.43 ± 3.29	49.58 ± 1.67	49.82 ± 1.88
EDV (ml)	0.34 ± 0.03	0.34 ± 0.02	0.34 ± 0.02	0.33 ± 0.03	0.35 ± 0.02	0.30 ± 0.02	0.32 ± 0.02	0.33 ± 0.03	0.31 ± 0.04	0.24 ± 0.02***†††
RVID (cm)	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.02*	0.21 ± 0.04***††	0.28 ± 0.04***†††

Results are presented as x±SD; IVSd: interventricular septum in diastole; LVIDd: left ventricular internal diameter in diastole; PWd: posterior wall in diastole; IVSs: interventricular septum in systole; LVIDs: left ventricular internal diameter in systole; PWs: posterior wall in systole EF: left ventricular ejection fraction; FS: Fractional shortening; EDV: left ventricular end diastolic volume; RVID: right ventricular internal diameter; *p < 0.05; **p < 0.01; ***p < 0.001 vs baseline within the same experimental group, †p < 0.05; ††p < 0.01; †††p < 0.001 vs. saline-treated control group

Table 2. Histomorphometric parameters of rat heart

Parameters	C1	C2	MCT	B6+FA	MCT+B6+FA
LV wall thickness ($\mu\text{m} \pm \text{SD}$)	1950.80 $\pm$ 227.33	2363.81 $\pm$ 346.21	1676.26 $\pm$ 208.07	2446.7692 $\pm$ 368.95	2453.48 $\pm$ 237.89 $\square$
RV wall thickness ($\mu\text{m} \pm \text{SD}$)	731.55 $\pm$ 131.26	835.48 $\pm$ 144.63	1229.63 $\pm$ 231.80*	914.70 $\pm$ 134.42	1346.06 $\pm$ 304.01 ^
IVS thickness ($\mu\text{m} \pm \text{SD}$)	1848.82 $\pm$ 144.96	2112.61 $\pm$ 296.05	1651.02 $\pm$ 293.95	2075.65 $\pm$ 388.79	2163.97 $\pm$ 580.50 $\square$
Dimension of cardiomyocyte of the LV free wall ($\mu\text{m} \pm \text{SD}$)	17.99 $\pm$ 1.90	22.50 $\pm$ 1.90	21.20 $\pm$ 1.55*	22.51 $\pm$ 1.88	21.73 $\pm$ 2.50

Results are presented as $x \pm \text{SD}$; LV – left ventricle; RV – right ventricle; IVS – interventricular septum; SD – standard deviation;

* – $P < 0.05$ versus C1 group, $\square$ – $P < 0.05$ versus MCT group, ^ – $P < 0.05$ versus B6+FA group

Table 3. Biochemical parameters in serum or plasma of the experimental animals

Groups/ parameters	C1	C2	MCT	B6+FA	MCT+B6+FA
‡hsTnT(nmol/L)	17 (16-25)	25 (19-37)	199 (58-251)*	24 (19-26)	163 (119-369) ^
°Hcy(μmol/L)	8.36 ± 2.48	10.19 ± 3.87	8.01 ± 1.08	10.73 ± 1.96	7.08 ± 2.69
°GLUC(mmol/L)	6.19 ± 0.63	7.17 ± 0.58	7.33 ± 1.20*	7.39 ± 0.70	6.92 ± 0.71
°UREA(mmol/L)	13.66 ± 1.96	9.90 ± 0.54	18.27 ± 6.97	9.66 ± 0.71	23.20 ± 12.08
°CREA(μmol/L)	43.90 ± 2.56	29.00 ± 1.76	47.18 ± 14.43	45.80 ± 2.30	56.88 ± 20.29
°CHL(mmol/L)	1.38 ± 0.15	1.45 ± 0.23	1.37 ± 0.24	1.67 ± 0.23	2.46 ± 1.32 □^
°HDL(mmol/L)	1.21 ± 0.13	0.64 ± 0.09	0.84 ± 0.26*	0.84 ± 0.11	1.14 ± 0.75
°TGL(mmol/L)	0.89 ± 0.16	0.77 ± 0.17	0.95 ± 0.33	0.91 ± 0.26	1.61 ± 1.15 □^
°AST(U/L)	343.20 ± 76.03	222.80 ± 49.56	352.91 ± 119.57	220.70 ± 33.24	325.00 ± 398.93
°ALT(U/L)	129.20 ± 25.47	69.50 ± 8.77	76.27 ± 28.34	75.30 ± 5.72	56.50 ± 41.62
°ALP(U/L)	497.90 ± 71.15	347.80 ± 70.38	391.55 ± 156.74*	434.90 ± 80.24	227.00 ± 65.33 □^
°TP(g/L)	62.20 ± 2.35	60.60 ± 1.96	52.55 ± 5.39*	59.70 ± 2.11	53.50 ± 5.40 ^
°ALB(g/L)	36.40 ± 0.84	30.40 ± 1.58	29.36 ± 2.87*	34.90 ± 1.20	24.00 ± 3.96
°URIC ACID(μmol/L)	74.40 ± 14.28	77.20 ± 14.11	85.45 ± 22.96	65.60 ± 9.82	78.25 ± 24.85
°α-AMY(U/L)	3560.75 ± 580.49	3097.4 ± 386.83	2530.00 ± 657.01*	1673.00 ± 226.58	3036.75 ± 459.92 □^
‡IL6 < 1.5(pg/mL)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
‡CRP < 2(mg/L)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
‡Fibrinogen < 1.8(g/L)	8 (100%)	8 (100%)	6 (75%)	8 (100%)	4 (50%)
‡vWF(IU)	156.00 ± 37.19	15.40 ± 0.89	227.65 ± 41.01*	14.00 ± 0.00	141.84 ± 115.84 ^

‡- Results are presented as percentile; °- Results are presented as x±SD; ‡- Results are presented as n (%)

* – P < 0.05 versus C1 group, □ – P < 0.05 versus MCT group, ^ – P < 0.05 versus B6+FA group

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160x87mm (300 x 300 DPI)

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