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Fisiopatologia da Hipertensão Arterial Pulmonar Infantil Induzida pela Monocrotalina

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Prof. Doutor Tiago Henriques-Coelho e
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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR
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Physiopathology of infantile pulmonary arterial hypertension induced by monocrotaline

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Abstract:
Introduction: Pediatric pulmonary arterial hypertension (PH) presents certain specific features, however there is a lack of experimental models to study the physiopathology of PH in this specific age group.
Aim: To characterize hemodynamic, morphometric and histological progression as well as expression of neurohumoral factors and regulators of cardiac transcription in an infantile model of PH induced by monocrotaline (MCT).
Methods: Eight-days-old Wistar rats were randomly injected with MCT (30mg/Kg, sc, *n*=95) or equal volume of saline solution (*n*=92). Different time points after injection were defined for analysis. Hearts and lungs were collected for morphometric characterization and stained with picrosirius red for assessment of the RV and LV collagen type I and type III ratio, RV collagen volume fraction (days 1, 3, 7, 14 and 21) and pulmonary vessels wall thickness (days 7, 14 and 21). mRNA quantification was undertaken for BNP, ET-1, HOP and Islet1 (days 1, 7 and 21). Animals were instrumented for biventricular hemodynamic recording on days 7, 14 and 21 after treatment.
Results: Animals treated with MCT at the 8th day of life presented RV hypertrophy since day 7 after MCT injection. There were no differences on the RV collagen volume fraction or collagen type I and type III ratio. Pulmonary vascular remodeling and PH were present on day 21, which were accompanied by an increased expression of BNP, ET-1, HOP and Islet1.
Conclusion: The model of MCT induced pediatric PH can be useful for physiopathological studies and to test new therapeutic targets in this age group.

Keywords: Pulmonary hypertension, Pediatric, BNP, Endothelin-1, HOP, Islet1.

Introduction

Pulmonary arterial hypertension (PH) is characterized by vascular remodeling of pulmonary arteries that lead to increased vascular resistance, elevated pulmonary arterial pressures and right ventricular (RV) hypertrophy and failure [1]. This serious progressive condition carries a poor prognosis if not identified and treated early, particularly in children. In fact, prior to the advent of the current treatment, idiopathic PH exhibited a prognosis with a 10 month median survival for children after diagnosis, compared to 2.8 years of global median survival [2]. Although further advances took place in the past decades [3-5], there is still no cure for this condition. The pathophysiology of infantile pulmonary hypertension remains less understood, mainly due to the particularities of pediatric PH [6] . Children with PH often have an exaggerated response of the pulmonary vascular bed to exercise and a greater vasoreactive response to hypoventilation compared with adults [7]. Furthermore, the rare reported cases of *spontaneous regression* of idiopathic PH have occurred in childhood [8-9] and it was shown that early corrective surgery is able to safeguard against the persistence or progression of structural changes in the pulmonary vascular bed in children with PH secondary to congenital heart defects [10].

The prognosis of PH is intimately related with RV hypertrophy and failure. The mechanisms of this progression are largely unknown. Many forms of adult cardiac hypertrophy and heart failure share the process of reactivation of genes that were expressed during embryonic development but that are normally silent or downregulated in the adult, known as “fetal gene program” [11]. In this regard, the regulators of cardiac transcription, represented by homeodomain only protein (HOP) and Islet1 may play an important role since they govern a number of cardiac-specific genes during cardiogenesis that may become reactivated in postnatal heart disease [11-12]. HOP is expressed by cardiac myocytes during gestation and in the adult. In the absence of HOP there is an imbalance between cardiomyocyte proliferation and differentiation with consequent abnormalities in cardiac morphogenesis. On the other side, transgenic mice that overexpress HOP develop severe cardiac hypertrophy, cardiac fibrosis, and premature death [13]. In the failing human myocardium, increased levels of myocardin mRNA are associated with a diminished HOP transcript content [14]. The LIM-homeodomain transcription factor Islet1 has been claimed as a marker of cardiac progenitors in postnatal rat, mouse and human myocardium [15]. During cardiogenesis, Islet1^{+} cells make a substantial contribution to the embryonic heart and Islet1-deficient mouse embryos lack an outflow tract and right ventricle [16]. When cocultured with neonatal myocytes, Islet1^{+} cells represent authentic, endogenous cardiac progenitors (cardioblasts) that differentiate into a

mature cardiac phenotype, with intact Ca^{2+}-cycling and the generation of action potentials. Because they are rare in the neonatal heart and even rarer or may not exist in the adult heart, these cells may not be considered analogous to satellite cells in skeletal muscle. Nevertheless, its role in providing myocyte reserve capacity in response to cardiac injury in early age stages may be hypothesized.

The aim of this study is to characterize hemodynamic, morphometric, histological and neurohumoral progression as well as expression of regulators of cardiac transcription, namely HOP and Islet1, in an infantile model of PH induced by monocrotaline (MCT).

Methods

Experimental Design

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the experiments were performed according to the Portuguese law on animal welfare. Wistar pups (males and females, Charles-River, Barcelona, Spain) were housed with their dam in a controlled environment under a 12:12 h light/dark cycle at a room temperature of 22ºC, with free supply of food and water. At day 8 after birth, rats randomly received a subcutaneous injection of monocrotaline (MCT, *n*=95; 30 mg/kg; Sigma Chemical, St. Louis, MO, USA) or an equal volume of vehicle (Ctrl, *n*=92; 1 mL/kg of saline).

In Ctrl and MCT-treated rats, hemodynamic studies were carried out on days 7 (MCT, *n*=5 and Ctrl, *n*=6) 14 (MCT, *n*=8 and Ctrl, *n*=6) and 21 (MCT, *n*=5 and Ctrl, *n*=6) after MCT/vehicle injection. Morphometric analysis was performed on days 1 (MCT, *n*=5 and Ctrl, *n*=5), 3 (MCT, *n*=5 and Ctrl, *n*=5), 7 (MCT, *n*=8 and Ctrl, *n*=7) 14 (MCT, *n*=8 and Ctrl, *n*=8) and 21 (MCT, *n*=5 and Ctrl, *n*=8) after MCT/vehicle injection. For histological studies, 4 animals were analyzed per group (MCT, *n*=20 and Ctrl, *n*=20). RV and left ventricle (LV) collagen type I and type III ratio and RV collagen volume fraction were carried out on days 1, 3, 7, 14 and 21 after injection and assessment of pulmonary vessels wall thickness on days 7, 14 and 21. For molecular analysis, samples of the RV were used on days 1 (MCT, *n*=10 and Ctrl, *n*=9), 7 (MCT, *n*=7 and Ctrl, *n*=5) and 21 (MCT, *n*=9 and Ctrl, *n*=7) after MCT/vehicle injection. The experimental protocol is summarized in Table 1.

Hemodynamic Studies

Seven, fourteen and twenty-one days after MCT/vehicle injection, the rats (MCT and Ctrl) were anesthetized by inhalation of a mixture of sevoflurane (4%) and oxygen, after a short anesthetic induction with ether, tracheostomized for mechanical ventilation (Harvard Rodent Ventilator model 683), that was adjusted for animals weight, and placed over a heating pad to prevent hypothermia. The heart was exposed through a median sternotomy, and the pericardium was widely opened. RV and LV pressures were measured with conductance catheters (Mikro-Tip® catheter transducers, 1F (size), 3 cm (length), ref. 842.000; Millar Instruments) inserted into the RV and LV cavities, respectively. After complete

instrumentation, the animal preparation was allowed to stabilize for 10 min. Hemodynamic recordings were made with respiration suspended at end expiration. The following parameters were derived by use of PVAN version 3.5 software: heart rate (HR), bpm, peak systolic RV and LV pressures (RV P_{max} and LV P_{max}), mmHg, peak rates of RV and LV pressure rise (dP/dT_{max}), ms, and pressure decline (dP/dT_{min}), ms, and time constant tau (τ), ms, calculated by Weiss method [regression of log(pressure) vs. time] as a measure of relaxation rate.

Morphometric Analysis

On days 1, 3, 7, 14 and 21 after MCT/Vehicle injection, animals were sacrificed by an intraperitoneally administered overdose of sodium pentobarbital (300 mg/kg). The hearts were excised and weighed. Under binocular magnification (x3.5), the RV free wall was dissected from the LV and weighed separately. Heart, RV, and LV plus septal (LV + S) weights were normalized to body weight. Additionally, RV weight was normalized to that of LV + S.

Histological Analysis

One, three, seven, fourteen and twenty-one days after MCT/vehicle injection, the rats (MCT and Ctrl) were sacrificed by an intraperitoneally administered overdose of sodium pentobarbital (300 mg/kg). Heart and lungs were exposed through a median sternotomy. Lungs were then distended by intratracheal infusion of 10% formaldehyde (at room temperature), after trachea isolation. In the end of the infusion, trachea was occluded and the heart and lungs were then removed *en bloc*. Five µm thick transverse-sections of paraffin-embedded, formalin-fixed specimens encompassing the heart and lungs from MCT and Ctrl groups were stained with picrosirius red, as previously described [17]. RV and LV of MCT and Ctrl groups were photographed using a polarized microscope (x400) on days 1, 3, 7, 14 and 21 (one section of each ventricle was analyzed per animal). Image-Pro Plus Version 6.0.0.260 software was used by two different blinded observers to determine the relative proportion between collagen type-I and type-III according to their different birefringences. Additionally, RV of MCT and Ctrl groups were photographed using a non-polarized microscope (x400) at days 1, 3, 7, 14 and 21 (five sections of the RV were analyzed per animal) for quantifying collagen volume fraction as previously described [18].

Using the same stained sections, lungs of MCT and Ctrl groups were photographed using a non-polarized microscope (x400) on days 7, 14 and 21. These photographs were analyzed regarding external diameter and medial thickness of pulmonary vessels with external diameter less than 100 µm (6 arteries/lung). Orthogonal intercepts were used to generate 8 random measurements of external diameter of the vessels (distance between the external laminas) and 16 random measurements of medial thickness of the vessels (distance between the internal and external lamina). For each artery, medial hypertrophy was expressed as follows: %wall thickness = [(medial thickness x 2)/(external diameter)] x 100.

mRNA Quantification by Real-Time RT-PCR

On days 1, 7 and 21 after MCT/vehicle injection, the rats (MCT and Ctrl) were sacrificed by an intraperitoneally administered overdose of sodium with pentobarbital (300 mg/kg). After tissue collection, RV samples were frozen in liquid nitrogen, and stored at -80ºC. Total RNA was extracted using Trizol (Invitrogen), according to established protocol. Concentration and purity was assayed in a spectrophotometer at 260 nm (Eppendorf). For relative quantification of specific mRNA levels, 100-150 ng of total mRNA from each sample was used in a Reverse Transcription- Polymerase Chain Reaction (RT-PCR) (10 µl total reaction volume) (RT reaction: 30 ng/mL random primers (Invitrogen 48190-011), 20 U/reaction of RNase inhibitor (Promega N2515), 40 U/reaction of reverse transcriptase (Invitrogen 18064-014), 0.5 mM dNTP mix (MBI Fermentas R0192), 1.9 mM $MgCl_2$ and 10 mM dTT.). cDNA yield was used as template for Real-Time PCR (LightCycler Roche) using SYBR Green (Quiagen), following manufacturer's instructions. Two replicas for each sample and for each gene were run simultaneously. Values obtained were averaged and normalized for GAPDH. Results were expressed as arbitrary units (AU) (set as the average value of each group, after normalization for GAPDH). Specific primers for the studied genes were designed in-house with the aid of software (Primer3): GADPH – fw 5'-TGCCACTCAGAAGACTGT GG-3' and rev 5'-GGATGCAGGGATGATGTTCT-3', BNP – fw 5'-GGACCAAGGCCCTACAAAAGA-3' and rev 5'-CAGAGCTGGGGAAAGAAGAG-3', ET-1 – fw 5'-CGGGGCTCTGTAGTCAAT GTG-3' and rev 5'-CCATGCAGAAAGGCGTAAAAG-3', HOP – fw 5'-GAGGCTCTCCATCCTTAGCC -3' and rev 5'- GGGTGCTTGTTGACCTTGTT -3' and Islet1 – fw 5'-AAGGACAAGAAACGCAGCAT -3' and rev 5'- CCATCATGTCTCTCCGGACT -3'.

Statistical Analysis

Statistical analysis was performed using SigmaStat 3.5 software and graphs were obtained with SigmaPlot10.0. Values were expressed as mean ± SEM. Differences in hemodynamic, morphometric, histological and genetic studies were evaluated by two-way ANOVA. When groups were statistically different, the Holm-Sidak Test was selected do perform the pairwise multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Morphometric analysis

Parameters related to somatic and cardiac growth are summarized in Table 2 and Fig. 1. Body weight significantly increased in both Ctrl and MCT groups since day 3. When compared with Ctrl group, MCT treated animals presented RV hypertrophy as expressed by significant increase in the ratio of RV to LV plus septum (LV+ S) weights since day 7 until day 21 (Fig. 1). Heart, RV and LV + S weights normalized for body weight showed significant increase since day 14 in MCT groups as compared to Ctrl groups.

Bi-ventricular hemodynamic parameters

Hemodynamic data are summarized in Table 3 and Fig. 2. Peak systolic pressure of the RV (RV P_{max}) was used to estimate PH. Pulmonary hypertension was present at day 21 after MCT injection when compared to Ctrl group (Fig. 2a). Ctrl groups presented no differences in RV P_{max} during the analyzed time points.

The contractility index dP/dt_{max} and the relaxation index dP/dt_{min} presented significant increase in the RV of the MCT group on day 21 when compared to Ctrl group (Fig. 2b and c). In Ctrl groups no differences were detected among the times points analyzed. There were no other differences in the hemodynamic parameters measured in RV. With regard to LV hemodynamics, no differences were detected between groups (Table 3).

Pulmonary vascular remodelling

There was a significant increase in wall thickness in MCT group as compared to Ctrl group on day 21 (Fig. 3). Both Ctrl and MCT groups presented increased in wall thickness of pulmonary vessels from day 7 to day 14.

Cardiac fibrosis

Picrosirius red-stained *lung* sections images acquired with a non-polarized microscope from MCT and Ctrl animals on days 1, 3, 7, 14 and 21 are depicted in Fig. 4.

Picrosirius red staining revealed no differences regarding RV collagen volume fraction (Fig. 5 and Table 4) or relative proportion between collagen type-I and type-III in both RV and LV (Fig. 6 and Table 4).

Neurohumoral activation

MCT treatment induced an increase in BNP and ET-1 mRNA levels in the RV at day 21 (Fig. 7a and b). In MCT groups, BNP expression was significantly higher on day 21 when compared to day 7 and day 1. Regarding to ET-1 expression, a significant increase in mRNA levels was identified between day 1 and day 21. In Ctrl groups, neither BNP nor ET-1 expression revealed differences among the time points analyzed.

Cardiac transcription factors

Concerning the expression of transcription factors in the RV myocardium, there was a significant increase in both HOP and Islet1 in MTC groups on day 21 as compared to Ctrl group. There were no other differences among the time points analyzed (Fig. 8a and b).

Discussion

In the present study, we used a MCT model of PH induced in Wistar rats during pediatric age to study the progression of the disease. Animals treated with MCT at the 8th day of life presented RV hypertrophy since day 7. In this model, myocardial hypertrophy was not accompanied by fibrosis. Medial hypertrophy of the pulmonary vessels and hemodynamic evidences of PH appeared on day 21, which were accompanied by an increase in BNP and ET-1 expression. We also describe for the first time an increase in myocardial mRNA levels of HOP and Islet1, transcription factors involved in cardiac development which can participate in cardiac hypertrophy.

Experimental MCT model was introduced more than 40 years ago [19]. The effect of MCT is thought to be due to its activation to the reactive MCT pyrrole by hepatic cytochrome P450 3A [20-21]. The short half-life of MCT toxic metabolite results in lesion of the pulmonary arteries endothelium [22]. In adult rats, MCT administration leads to pulmonary vasculature remodeling (medial hypertrophy and muscularization of peripheral arteries) with RV hypertrophy [23] and failure, reproducing many characteristics of the human PH. Pulmonary lesion severity is dose-dependent: high doses cause pulmonary edema and death, while low doses induce pulmonary arterial hypertension, hypertrophy of the arterial walls, endothelial proliferation and perivascular inflammation [24]. In 1985 Todd et al [23] described an infantile model of PH induced by a MCT injection (60mg/Kg) in eight days-old Sprague-Dawley rats. Rats injected with MCT during infancy had a normal alveolar development but developed extension of muscularization into peripheral arteries, medial hypertrophy of muscular arteries and RV hypertrophy. In the present study, we used Wistar rats that were initially injected with 60 mg/Kg of MCT. However, we had a 100% mortality 21 days after administration and the animals developed severe cachexia, jaundice, ascites and pleural effusion. The use of different rat strains with probable different MCT sensitivity might explain, at least partially, this finding. In this regard, a dose of 50 mg/Kg was also tested, but was associated as well with 100% mortality. A dose of 30 mg/Kg was finally tested with an acceptable mortality. Based on these preliminary data, a dose of 30 mg/Kg of MCT was used in the present study.

The development of PH induced by MCT was evaluated during different time points in order to identify the beginning of the hemodynamic, morphometric and molecular changes. We found that pulmonary vascular remodeling and pulmonary hypertension are late events, present only 21 days after

MCT injection. These results are similar to the MCT adult model where the increase in RV peak systolic pressure starts approximately 3 weeks after injection [25]. Classically, the development of PH is accompanied by neurohumoral activation. Our group previously showed in MCT adult model an activation of ET-1 and ACE expression at myocardial level [26-27]. Similarly, in the present study we also found an increase in mRNA levels of ET-1, 3 weeks after MCT injection, concomitant with the development of systolic and diastolic changes in the hemodynamic evaluation. Several studies suggested that cardiac ET-1 contributes to the progression of cardiopulmonary alterations in rats with MCT-induced pulmonary hypertension [28-29]. Additionally, there is evidence for the beneficial effects of ET-1 receptors antagonists in MCT-induced PH [30]. Therefore, we could hypothesize that ET-1 signalling is an important pathway in pediatric PH pathophysiology. BNP is a marker of ventricular volume and/or pressure overload and is correlated with mean pulmonary artery pressure and pulmonary vascular resistance in patients with PH [31-32]. Our finding of increased BNP expression in the RV confirms the presence of overload in this cardiac chamber. Accordingly, this overexpression occurs at day 21, when medial hypertrophy of pulmonary vessels is maximally increased in MCT group. During progression of infantile PH, we found that RV hypertrophy is an early event, present since 7 days after MCT injection. This result seems to be specific for this age group because in adult model RV hypertrophy is a late finding, only present at 3 or 4 weeks after MCT injection [23]. As myocardial hypertrophy was not accompanied by cardiac fibrosis, we could hypothesize that myocardium from young rats is capable of a compensatory cardiac growth. To explore this possibility, we analyzed two transcription genes involved in heart development that might be activated during cardiac adaptation to overload. HOP expression is first seen in mesodermal precursors of the cardiac muscle during early to mid-gestation. Immediately after birth, HOP is most strongly expressed in the endocardium and interventricular septum with less robust expression throughout the rest of the myocardium [33]. Transgenic mice engineered to overexpress HOP develop cardiac hypertrophy. HOP was considered to function as a repressor of anti-hypertrophic transcriptional program that functions in adult cardiomyocytes by shifting the balance between pro- and anti-hypertrophic pathways [13]. Our findings of increased RV expression of HOP in MCT group on day 21, in the presence of marked RV hypertrophy, suggests that HOP pathway might be implicated in cardiac hypertrophy.

Besides its role in heart developmental, Islet1 has recently been considered a marker of cardiac progenitors in postnatal rat. After birth, an average of 500 to 600 Islet1^{+} cardioblasts is still detectable in

the rat myocardium. Because their organ distribution corresponds to contributions of Islet1+ embryonic precursors, it was suggested that these cells are remnants of the fetal progenitor population. Phenotipically, besides Islet1, these cardioblasts express GATA-4 and Nkx2.5 and lack transcripts of mature myocytes. Because the contribution of exogenous and endogenous progenitor cardiac cells has been increasingly addressed in cardiac physiopathology [34-36], we hypothesized a contribution of cardiac progenitors marked by Islet1 in infantile pulmonary hypertension. Accordingly to the described rare frequency of these cells in postnatal period, we found low levels of mRNA expression in Ctrl groups among all time points analyzed. Our finding of increased mRNA levels of Islet1 in MCT group compared to Ctrl group on day 21 supports the idea of a possible activation of these progenitor cardiac cells by an overload stimulus and may represent a compensatory response leading to cardiomyocytes regeneration.

In conclusion, we characterized the morphometric, histological and hemodynamic changes induced by MCT in an infantile model of PH. This model is accompanied by an increase in neurohumoral factors and an activation of fetal transcription genes.

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Legends

Figure 1 Right ventricular (RV) hypertrophy during the progression of PH. Ratio of RV and left ventricle plus septum (LV + S) weights on days 1, 3, 7, 14 and 21 after injection. Ctrl, control groups; MCT, monocrotaline groups; D1 to D21, 1 day to 21 days after MCT/vehicle injection. $p<0.05$: * *vs* Ctrl of the corresponding day; # vs D1.

Figure 2 Right ventricular systolic and diastolic hemodynamic parameters. Right ventricular peak systolic pressure (RV P_{max}) (**a**) peak rate of RV pressure rise (dP/dT_{max}) (**b**) and peak rate of RV pressure decline (RV dP/dt_{min}) (**c**) on days 7, 14 and 21 after monocrotaline (MCT) or vehicle injection (Ctrl). D7 to D21, 7 to 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; † vs D14; ‡ vs D7.

Figure 3 Pulmonary vasculature wall thickening during progression of PH expressed by the percentage of wall thickness of pulmonary vessels (diameter inferior to 100 µm) among monocrotaline (MCT) and control (Ctrl) groups, 7, 14 and 21 days after injection. D7 to D21, 7 to 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; † vs D14; ‡ vs D7.

Figure 4 Representative examples of pulmonary vessels (picrosirius red-stained *lung* sections images acquired with a non-polarized microscope). Ctrl, control groups; MCT, monocrotaline groups; D1 to D21, 1 to 21 days after MCT/vehicle injection.

Figure 5 Representative examples of right ventricular fibrosis (picrosirius red-stained *right ventricle* sections images acquired with a non-polarized microscope). Ctrl, control groups; MCT, monocrotaline groups; D1 to D21, 1 to 21 days after MCT/vehicle injection.

Figure 6 Representative examples of right ventricular expression of collagen type I and type III (picrosirius red-stained *right ventricle* sections images acquired with a polarized microscope showing collagen different birefringences). Ctrl, control groups; MCT, monocrotaline groups; D1 to D21, 1 to 21 days after MCT/vehicle injection.

Figure 7 *Right ventricular* BNP (**a**) and ET-1 (**b**) mRNA expression. Monocrotaline (MCT) and control (Ctrl) groups on days 1, 7 and 21. Results are expressed as arbitrary units (AU) after normalization for GADPH. D1, D7 and D21, 1, 7 and 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; ‡ vs D7; # vs D1.

Figure 8 *Right ventricular* HOP (**a**) and Islet1 (**b**) mRNA expression. Monocrotaline (MCT) and control (Ctrl) groups on days 1, 7 and 21. Results are expressed as arbitrary units (AU) after normalization for GADPH. D1, D7 and D21, 1, 7 and 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; ‡ vs D7; # vs D1.

Table1. Time points of hemodynamic, morphometric, histological and Real-Time RT-PCR analysis

	D1	D3	D7	D14	D21
Hemodynamic Studies	–	–	✓	✓	✓
Morphometric Analysis	✓	✓	✓	✓	✓
Histological Analysis:					
Collagen type I and type III ratio	✓	✓	✓	✓	✓
Collagen volume fraction	✓	✓	✓	✓	✓
Pulmonary vessels wall thickness	–	–	✓	✓	✓
mRNA (Real-Time RT-PCR)	✓	–	✓	–	✓

D1 to D21, 1 to 21 days after MCT/vehicle injection.

Table 2. Somatic and cardiac growth at different time points

	D1	D3	D7	D14	D21
Ctrl (*n*)	5	5	7	8	8
BW, g	20.34±2.04	21.12±2.04	30.63±1.73§#	47.75±1.62‡§#	84.10±1.62†‡§#
Heart weight/BW, g/kg	5.25±0.34	5.24±0.34	5.23±0.28	6.03±0.27	4.48±0.27†
RV weight/BW, g/kg	1.08±0.14	1.40±0.14	1.43±0.12	1.33±0.11	0.98±0.11
LV + S weight/BW, g/kg	3.04±0.13	2.99±0.13	3.18±0.11	3.44±0.10	2.72±0.10†‡
RV weight/LV + S weight, g/g	0.36±0.04	0.47±0.04	0.45±0.03	0.39±0.030	0.36±0.03
MCT (*n*)	5	5	8	8	5
BW, g	21.04±2.04	22.84±2.04	31.09±1.62§#	44.76±1.62‡§#	82.78±2.04†‡§#
Heart weight/BW, g/kg	4.99±0.34	5.11±0.34	5.66±0.27	7.52±0.27*‡§#	7.00±0.37*‡§#
RV weight/BW, g/kg	1.06±0.14	1.54±0.14	1.72±0.11#	2.31±0.11*‡§#	2.38±0.14*‡§#
LV + S weight/BW, g/kg	2.81±0.13	2.84±0.13	3.05±0.10	3.97±0.10*‡§#	3.54±0.13*†‡§#
RV weight/LV + S weight, g/g	0.38±0.04	0.54±0.04#	0.57±0.03**#	0.58±0.03**#	0.67±0.04**#

Data are given as mean ± SEM. RV, right ventricle; LV + S, left ventricle plus septum; Ctrl, Control groups; MCT, monocrotaline groups; BW, body weight; D1 to D21, 1 to 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; † vs D14; ‡ vs D7; § vs D3; # vs D1.

Table 3. Bi-ventricular hemodynamic at different time points

	D7		D14		D21	
	MCT	Ctrl	MCT	Ctrl	MCT	Ctrl
n	5	6	8	6	5	6
HR, bpm	385.27±17.19	376.42±16.46	360.14±15.23	382.18±17.19	396.18±17.19	410.17±16.46
RV						
P_{max}, mmHg	36.25±5.49	44.96±5.01	27.94±4.34	27.94±5.49	56.70±5.49*‡	28.52±5.01
dP/dt_{max}, mmHg/s	1654.20±317.40	2164.83±289.74	1625.88±250.92	1484.67±289.74	2713.20±317.40*†‡	1419.83±289.74
EDP, mmHg	5.45±2.13	7.86±1.95	8.10±1.69	4.29±1.95	5.42±2.13	5.38±1.95
dP/dt_{min}, mmHg/s	-1831.80±357.88	-1665.50±326.69	-1305.88± 282.92	-1269.33± 326.69	-2909.40±357.88*†	-1246.33±326.69
τ, ms	15.13±3.51	20.11±3.20	19.64±2.77	15.00±3.20	13.16±3.51	12.18±3.51
LV						
P_{max}, mmHg	61.68±7.34	68.03±7.34	71.87±7.34	69.78±8.05	86.06±7.34	76.18±7.34
dP/dt_{max}, mmHg/s	3043.67±638.50	3288.00±638.50	3377.00±638.50	3890.40±699.44	4636.00±638.50	4692.67±638.50
EDP, mmHg	7.74±2.016	4.78±2.02	6.94±2.02	6.12±2.209	7.80±2.02	6.28±2.02
dP/dt_{min}, mmHg/s	-3386.00±795.10	-3486.00±795.10	-2937.50±795.10	-3476.60±870.99	-4567.00±795.10	-5054.00±795.10
τ, ms	13.15±2.08	11.63±2.08	14.92±2.08	12.35±2.28	13.43±2.08	12.03±2.08

Data are given as mean ± SEM. HR, heart rate; RV, right ventricle; P_{max}, peak systolic pressure; dP/dt_{max} and dP/dt_{min}, peak rate of ventricular pressure rise and fall; EDP, end-diastolic pressure; LV, left ventricle MCT, monocrotaline groups; Ctrl, control groups; D7 to D21, 7 to 21 days after MCT/vehicle injection. $p<0.05$: * vs Ctrl of the corresponding day; † vs D14; ‡ vs D7.

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Table 4. Collagen volume fraction and collagen type-I/type-III ratio of right and left ventricle at different time points

	D1		D3		D7		D14		D21	
	Ctrl	MCT	Ctrl	MCT	Ctrl	MCT	Ctrl	MCT	Ctrl	MCT
RV collagen volume fraction	3.46± 0.38	5.17± 0.38	1.54± 0.44	2.85± 0.38	3.01± 0.38	2.99± 0.46	2.58± 0.40	4.83± 0.40	6.43± 0.56	3.28± 0.44
RV Collagen type-I/type-III	0.48 ±1.61	1.20 ±1.61	1.46 ±1.40	0.61 ±1.25	2.06 ±1.40	0.59 ±1.61	0.83 ±1.40	2.80 ±1.40	3.11 ±1.49	1.64 ±1.40
LV Collagen type-I/type-III	0.20 ±2.51	1.50 ±2.51	1.49 ±2.17	0.68 ±1.94	0.90 ±2.17	0.25 ±2.51	0.24 ±2.17	0.59 ±2.17	1.05 ±2.32	2.83 ±2.17

Data are given as mean ± SEM, *n*=4 animals per group. RV, right ventricle; LV, left ventricle MCT, monocrotaline groups; Ctrl, control groups; D1 to D21, 1 to 21 days after MCT/vehicle injection.

Figure 1

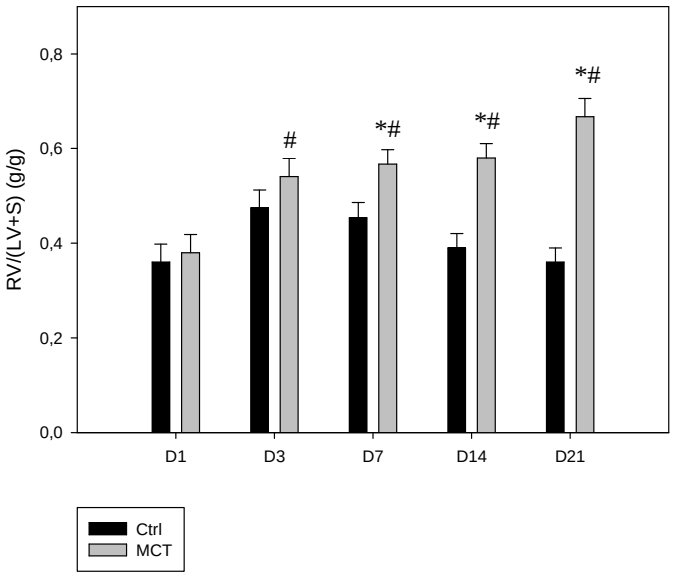
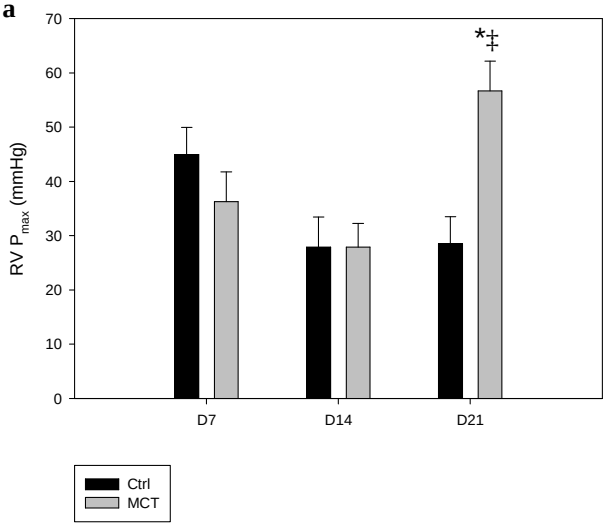
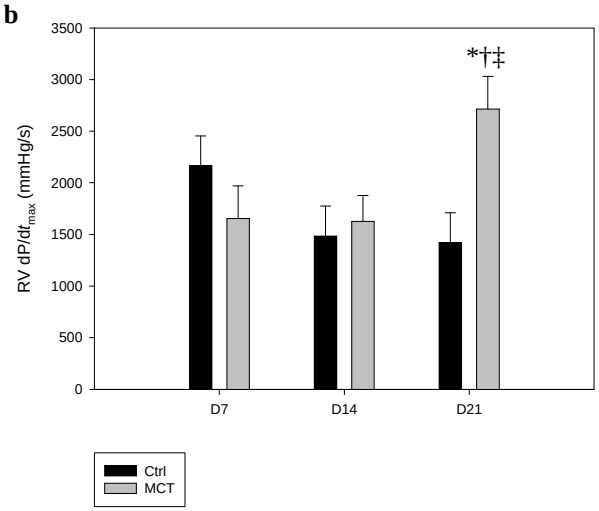


Figure 2

a


RV P_{max} (mmHg)
70
60
50
40
30
20
10
0
*‡
D7
D14
D21
Ctrl
MCT

b


RV dP/dt_{max} (mmHg/s)
3500
3000
2500
2000
1500
1000
500
0
*†‡
D7
D14
D21
Ctrl
MCT

c

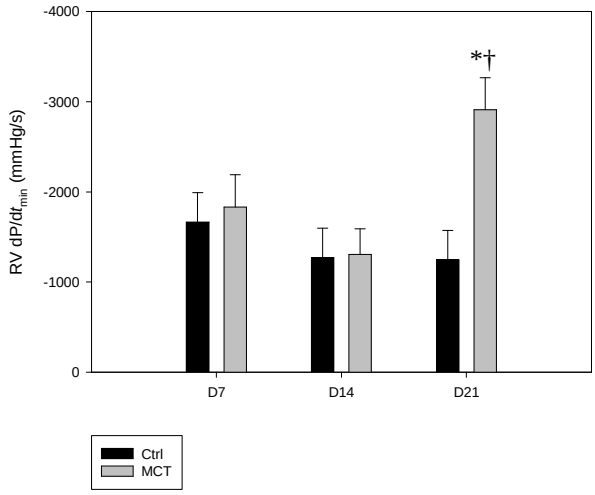

RV dP/dt_{min} (mmHg/s)
-4000
-3000
-2000
-1000
0
*†
D7
D14
D21
Ctrl
MCT

Figure 3

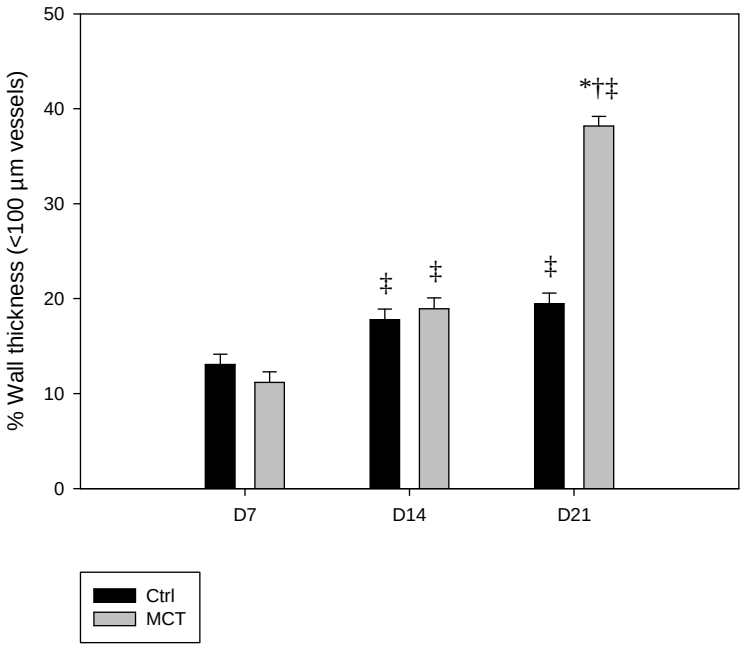

50
40
30
20
10
0
% Wall thickness (<100 µm vessels)
‡
‡
‡
*†‡
D7
D14
D21
Ctrl
MCT

Figure 4

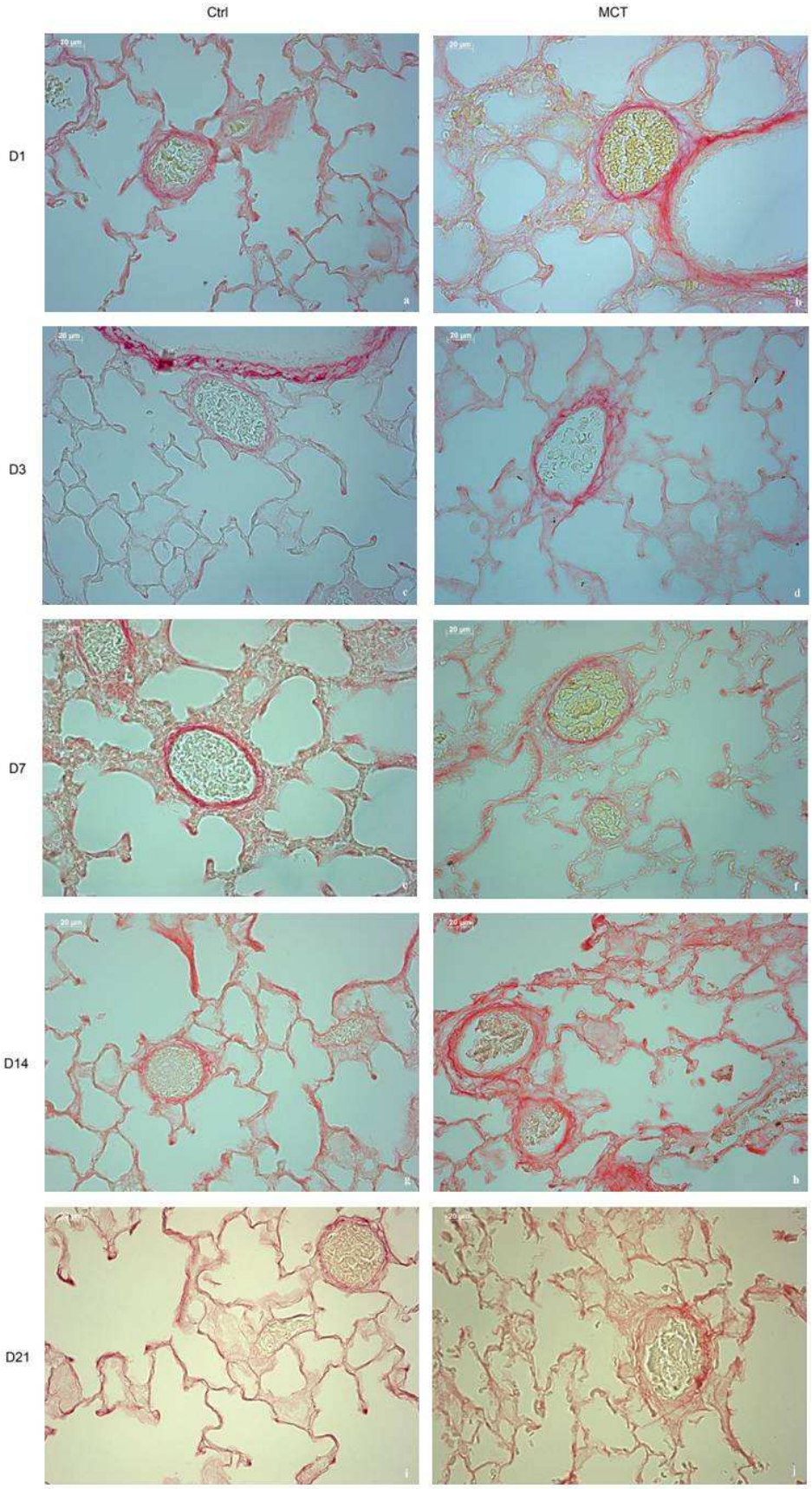


Figure 5

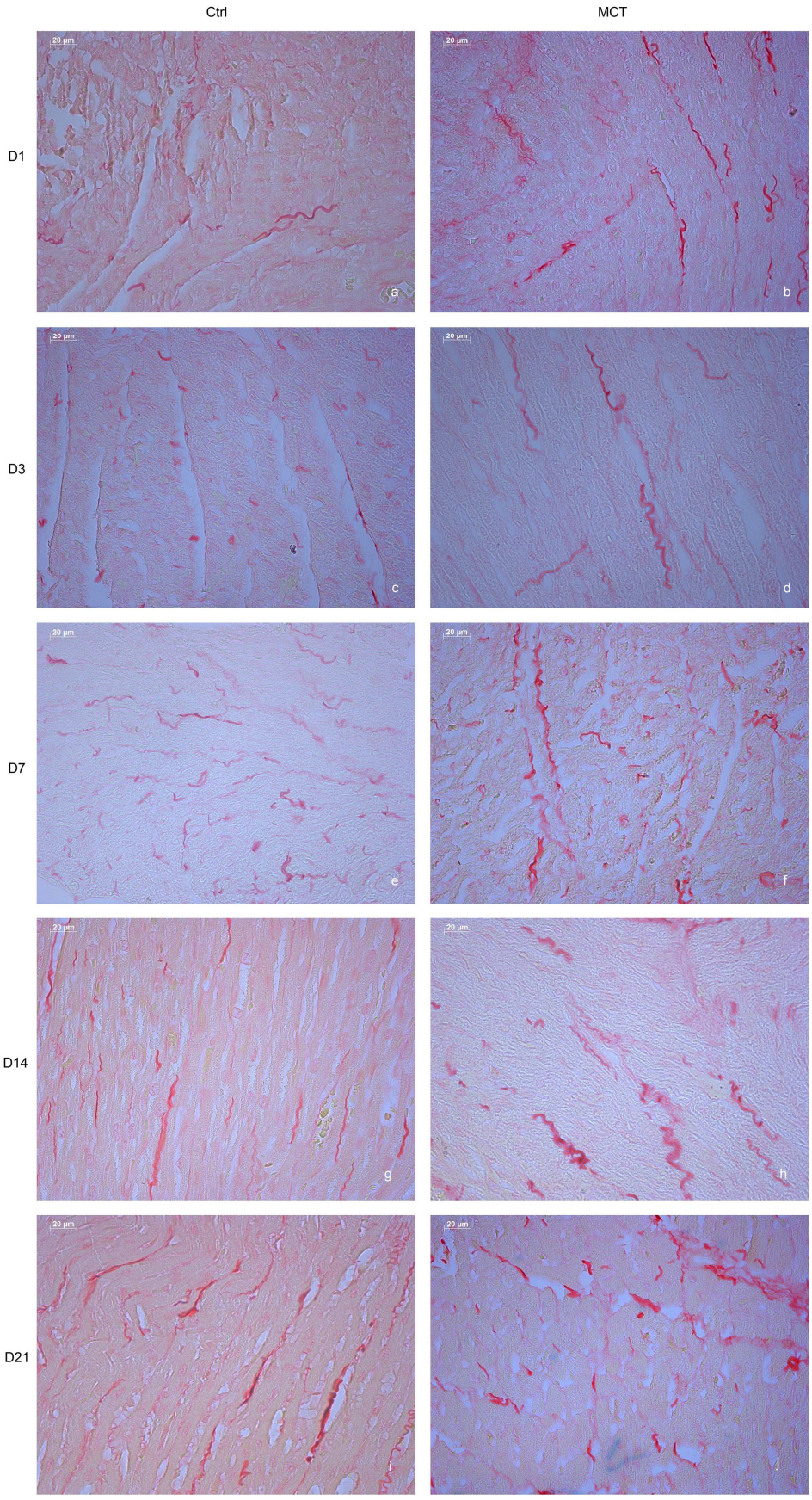


Figure 6

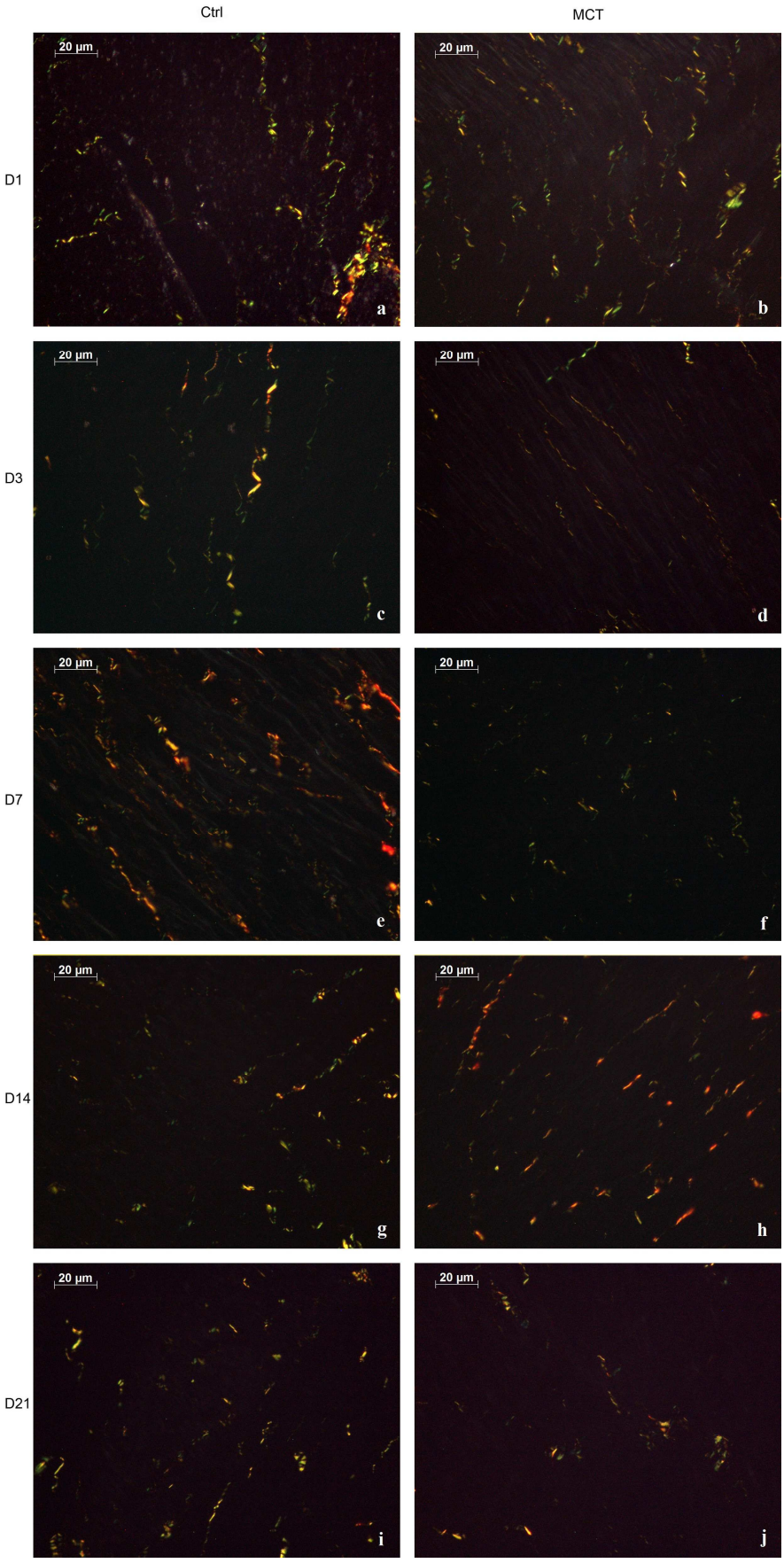
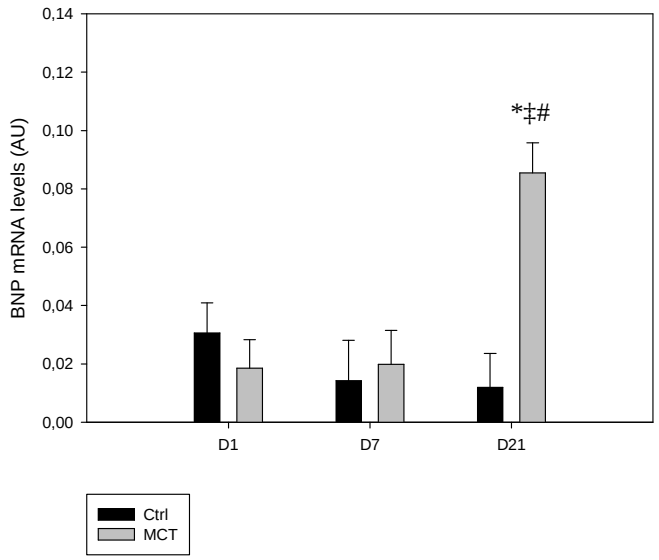


Figure 7

a


0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
BNP mRNA levels (AU)
*‡#
D1
D7
D21
Ctrl
MCT

b

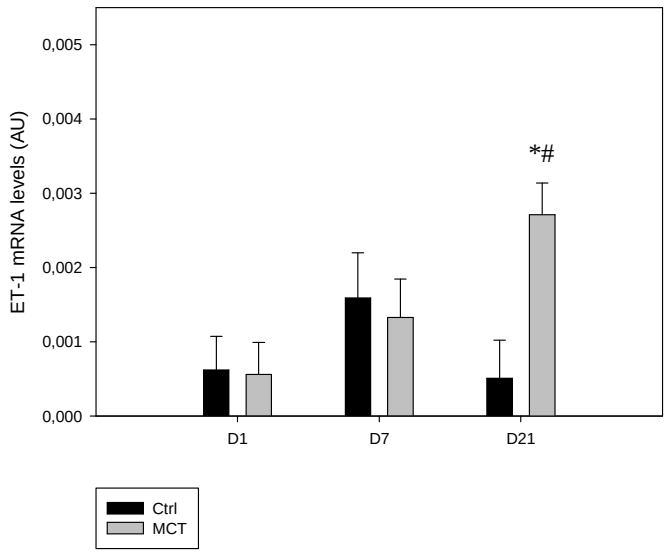
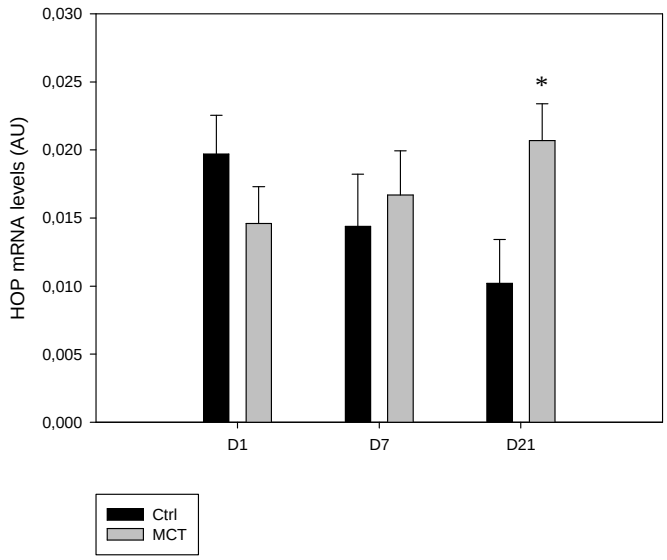
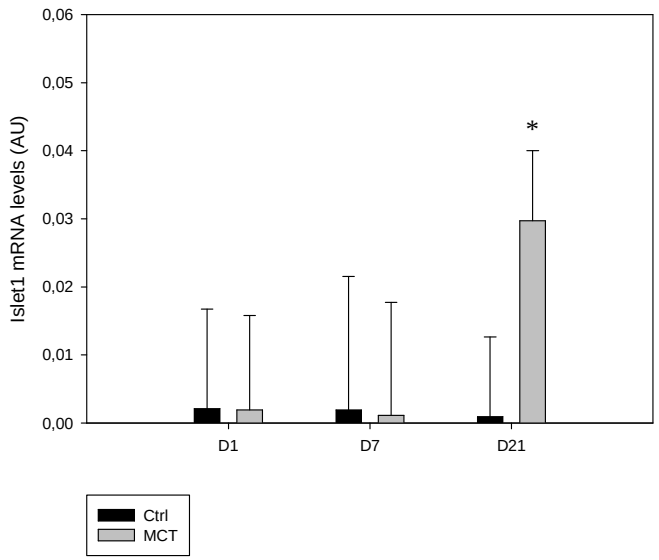

0,005
0,004
0,003
0,002
0,001
0,000
ET-1 mRNA levels (AU)
*#
D1
D7
D21
Ctrl
MCT

Figure 8

a


0,030
0,025
0,020
0,015
0,010
0,005
0,000
HOP mRNA levels (AU)
*
D1
D7
D21
Ctrl
MCT

b


0,06
0,05
0,04
0,03
0,02
0,01
0,00
Islet1 mRNA levels (AU)
*
D1
D7
D21
Ctrl
MCT