PROGESTERONE INHIBITS VASCULAR REMODELING AND ATTENUATES MONOCROTALINE-INDUCED PULMONARY HYPERTENSION IN ESTROGEN-DEFICIENT RATS

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A b s t r a c t: Pulmonary arterial hypertension (PH) is predominantly a disease of young females. Yet, little is known regarding the effects of female sex hormones in PH. Female rats develop less severe PH compared to male rats, and ovariectomy (OVX) exacerbates PH. Although OVX rats treated with estradiol develop less severe disease, the role of progesterone in OVX-induced exacerbation of disease has not been examined. Progesterone was shown to dilate pulmonary vessels and to inhibit proliferation of endothelial and vascular smooth muscle cells. Therefore, we hypothesized that progesterone may confer protective effects in experimental PH. A total of 30 female rats were ovariectomized and OVX rats were randomly administered either saline (OVX-Control group, n = 7), monocrotaline (60mg/kg i.p.; OVX-MCT group; n = 12), or MCT plus progesterone (30μg/kg/h via osmotic minipumps; OVX-MCT+P group; n = 11). After 32 days animals were instrumented for *in situ* (open chest) measurements of right ventricle (RV) peak systolic (RVSP) and end diastolic (RVEDP) pressures, and tissue samples were obtained for morphometric and histological analysis. Administration of MCT elevated RVSP (22.2 ± 1.1 vs. 46.7 ± 2.4 mmHg) and RVEDP (1.51 ± 0.86 vs. 11.9±2.2 mmHg), increased RV/left ventricle + septum (RV/LV+S) ratio (0.256 ± 0.010 vs. 0.582 ± 0.033, OVX vs. OVX-MCT), and induced media hypertrophy of small size pulmonary arteries. In ovariectomized pulmonary hypertensive rats, treatment with progesterone attenuated the severity of disease (OVX-MCT+P group: RVSP = 36.6 ± 2.3 mmHg; RV/LV+S = 0.468 ± 0.025; RVEDP = 7.5 ±1.5 mmHg), attenuated vascular remodeling (media % index: 28.2 ± 1.1 vs. 34.2 ± 1.3), and reduced mortality (9% vs. 25%; OVX-MCT+P vs. OVX-MCT).

This study provides the first evidence that in estrogen-deficient rats, progesterone has protective effects in MCT-induced PH. Further evaluation of the role of progesterone and its interaction with estrogens in pulmonary hypertension is warranted.

Key words: Pulmonary Hypertension, Progesterone, Estrogens, Vascular remodeling.

Introduction

Primary pulmonary hypertension is predominantly a disease of females with a female-to-male ratio of 1.7 : 1 [1, 2]. Yet little is known regarding the effects of female sex hormones in pulmonary hypertension. When exposed to chronic hypoxia or toxin monocrotaline, female animals develop less severe pulmonary hypertension, estrogen deficiency (i.e. ovariectomy) exacerbates pulmonary hypertension, and treatment with estradiol attenuates the disease [3–7]. Recently we have shown that ovariectomy worsened the disease, whereas treatment with 2-methoxyoestradiol (a major non-estrogenic metabolite of estradiol) prevented the worsening of the disease and eliminated high mortality [8]. This suggests that, in estrogen-deficient pulmonary hypertensive animals, in addition to estradiol its metabolites may also have beneficial effects. Although the beneficial effects of estradiol in pulmonary hypertensive estrogen-deficient animals are well established, the involvement of progesterone in the OVX-induced exacerbation of disease cannot be ruled out. However, the involvement of progesterone in the development of pulmonary hypertension has not been examined.

The presence of progesterone receptors has been reported in intact human endothelial cells (ECs) and in modified ECs within plexiform lesions in humans with PH [9, 10]. Natural progesterone inhibits proliferation of ECs by causing an arrest in the G1 phase of the cell cycle. This effect involves a reduction in cyclin-dependent kinase activity, and the altered expression of cyclin E and A in accordance with G1 arrest [11]. Progesterone receptors are also expressed in vascular smooth muscle cells (VSMCs; 12–14), and progesterone inhibits VSMCs proliferation [15–17]. The antiproliferative effect occurs at physiological concentrations (5–500 nM) in a dose-dependent manner and is blocked by the progesterone receptor antagonist RU 486 [16]. The antigrowth effect is paralleled by reduction in cyclin A, cyclin E, and cyclin-dependent kinase (CDK) 2, and an increase in CDK-inhibitory proteins p21 and p27 [17]. Finally, progesterone exerts vasodilatatory properties in different vascular beds, including the dilation of pulmonary vessels in rats and rabbits [18, 19].

Therefore, we hypothesized that progesterone may confer protective effects in experimental pulmonary hypertension and we examined the effects of progesterone on the development of pulmonary hypertension in ovariectomized rats.

Material and methods

A total of thirty female Sprague Dawley rats (253 ± 5 g) were housed at 22 °C, 45% relative humidity and 12-hour light/dark cycles. the animals had free access to water and were fed commercial lab food (Pro Lab RHM 3000 rodent diet, PMI Nutrition, Inc, St Louis, MO). Experimental protocols were approved by the University of Pittsburgh Animal Care and Use Committee and all experiments were conducted in accordance with the University guidelines for animal welfare. Under halothane anaesthesia, the animals underwent bilateral ovariectomy using the flank approach, and successful removal of ovaries was confirmed by measuring uterus weight at autopsy. Animals were randomly assigned to receive intraperitoneal injection of either vehicle (10 ml/kg ml 1N HCl neutralized with 1.0 N NaOH and diluted with distilled water; OVX-Control group; n = 7) or monocrotaline (MCT 60 mg/kg; MCT group n = 12,). Eight hours later, a subset of MCT animals (n = 9) was implanted with osmotic minipumps (model 2ML4, Alzet, Palo Alto, CA) delivering progesterone (30 μg/kg/hour, MCT + P group). All the other animals were implanted with minipumps delivering the vehicle (polyethylene glycol 400, 2.5 μl/hour). Thirty two days after administration of MCT, animals were anaesthetized (pentobarbital, 45mg/kg i.p.) and instrumented for measurement of systemic blood pressure and right ventricular peak systolic pressure (RVPSP), end diastolic pressure (EDP) and Tau (τ, a time constant of the isovolumetric relaxation of the right ventricle). Briefly, a PE-240 polyethylene catheter was inserted into the trachea to facilitate breathing and a PE-50 catheter was inserted into the left carotid artery and connected to a digital blood pressure analyzer (BPA, Micro-Med. Inc., Louisville, KY) for continuous measurements of systolic, diastolic and mean arterial blood pressure and heart rate. The rats were then mechanically ventilated (Harvard Rodent Ventilator, Model 683, Harvard Apparatus, MA) using constant breath rate (50/min) and tidal volume (1.0 ml). Next, the thorax was opened, and the right heart was punctured with a 23-gauge needle attached to a PE-50 line and Heart Performance Analyzer (HPA-200 τ, Micro-Med. Inc., Louisville, KY). After a 20-minute stabilization period, RVPSP, right ventricle EDP, and Tau, were recorded for 20 minutes at 1-minute intervals, and average values for parameters of right ventricular performance were calculated.

The animals were euthanatized by anesthetic overdose, and heart and lungs were dissected and weighed. The ratios of wet weights of heart and lung to body weight (BW) were calculated (H/BW and LV/BW, respectively). The right ventricle (RV) free wall was separated from the left ventricle and the septum (LV+S) to determine the wet weight, the RV to body weight ratio (RV/BW), the LV+S to body weight ratio (LV+S/BW), and the RV to LV+S weight ratio (RV/LV+S, Fulton Index; 20).

Since mortality was higher in MCT animals compared to MCT-P group, these animals had lived for a shorter period of time, and consequently had less time to fully develop RV hypertrophy. Therefore, we calculated the rate of progression of RV hypertrophy. The average RV/LV+S index for the OVX-Control group was subtracted from individual values for each animal in MCT and MCT+P groups, and absolute increase in Fulton index (RV/LV+S ratio) was calculated. Next, percent increase in Fulton index was divided by days into experiment (%/day) and this parameter was used as an indicator of the progression of RV hypertrophy.

The lungs were perfused *via* the trachea with 10% buffered formalin under constant low pressure (25 mmHg), and immersed in 10% buffered formalin for at least 72 hours before being embedded in paraffin. Four-micrometer serial tissue sections from formalin-fixed, paraffin-embedded lungs were dewaxed and stained with H&E and Masson's trichrome for histological and morphometric assessment. To examine the pulmonary vascular remodeling, measurements of media thickness, and media and adventitia surface were conducted using an Image Analyzing System (Diagnostic Instruments, Inc., Sterling Heights, MI) that included a SPOT RT Camera installed on a NIKON Eclipse 50 light microscope and a specialized computer software programme (SPOT Software, Version 4.1). Briefly, after calibrating each objective, measurements were done ($\times$ 40 magnification) on five cross-sectioned pulmonary artery branches with 50–250 μ in diameters. The measurements of the thickening vascular wall and media of pulmonary arteries were taken in the peripheral lung fields at approximately equal distances from the pleural lining. For each blood vessel, two rectangular diameters and their four respective media were measured, and averages of four individual values of media thickness and media % index were calculated. The media % index was calculated as $2 \times$ media/diameter $\times 100$.

All data are presented as mean $\pm$ S.E.M. Statistical analysis was performed using the Number Cruncher Statistical software programme (Kaysville, Utah). Group comparisons were performed by a one-factor analysis of variance (1-F ANOVA), followed by the Fisher = s LSD post-hoc comparison test. The probability value of $p < 0.05$ was considered statistically significant.

Results

A single injection of MCT induced pulmonary hypertension, right ventricular failure and hypertrophy, and resulted in 25% mortality. Monocrotaline significantly reduced body weight (Table 1) and, therefore, morphometric para-

meters were normalized by body weight. Ovariectomized controls, pulmonary hypertensive animals and diseased animals treated with progesterone (OVX-Control, MCT and MCT+P group, respectively) did not differ in regard to uterus weight (Table 1). However, in all three experimental groups uterus weight was significantly lower than uterus weight (76 ± 7 mg, n = 7) in age-matched intact female rats not included in the study, confirming the successful removal of ovaries (Table 1). Experimental groups also did not differ in regard to blood pressure and heart rate (data not shown). Significantly increased RVPSP, right ventricular EDP and Tau were detected in the MCT group (Figure 1), indicating that in addition to pulmonary hypertension the MCT rats had developed right ventricular systolic and diastolic dysfunction.

Treatment with progesterone significantly reduced RVPSP, improved right ventricular systolic function (i.e. reduced right ventricular EDP), and had no effects on the impaired isovolumetric relaxation of the right ventricle, i.e. Tau time constant (Figure 1). Progesterone reduced MCT-induced increase in lungs weight and had no effect on MCT-induced changes in body and heart weights (Table 1). No difference was detected among all three experimental groups in regard to weight of the left ventricle plus septum (Table 1). However, progesterone significantly reduced the MCT-induced increase in RV weight and RV/LV+S ratio (Figure 2), suggesting attenuation of isolated RV hypertrophy in pulmonary hypertensive animals by progesterone. Progesterone also reduced the %/day increase in the Fulton index, suggesting reduced progression of RV hypertrophy (Figure 3).

Histopathological analysis revealed marked media hypertrophy and adventitia widening in pulmonary hypertensive animals, as evidenced by increased media thickness, media % index, (Figure 4) and augmented media and adventitia surface area and wall/lumen and media/lumen ratio in small size pulmonary arteries (Figure 5b, Table 2). Progesterone attenuated media hypertrophy (Figure 5c, Table 2), and had no effects of MCT-induced adventitia expansion in MCT pulmonary hypertensive animals (Table 2). Finally, reduced pulmonary hypertension and vascular remodeling in progesterone-treated animals was associated with reduced mortality (9% vs. 25%, MCT+P vs. MCT group).

Табле 1

Body weight, and lung, heart, left ventricle plus septum (LV+S) and uterus wet weight, in ovariectomized controls (OVX) and in OVX monocrotaline pulmonary hypertensive rats receiving vehicle (MCT) or progesterone via osmotic minipumps for 32 days (MCT-Progesterone); *-p < 0.05 vs. OVX control; § – p < 0.5, vs. uterus weight of 76 ± 7 mg in age-matched intact female rats not included in the study, n = 7

Телесна тежина, и влажна тежина на бели дробови, срце, лева комора со септум (LV+S) и матка, кај овариектомизирани контроли (OVX) и кај овариектомизирани монокроталински белодробно хипертензивни стаорци кои добиле раствор (MCT) или прогестероне преку осмотски минипумпи во текот на 32 дена вклучени во студијата, n = 7

Group	Body Weight (BW) g	Heart		Lung		LV+ S g	Uterus § mg
		g	g/kg BW	g	g/kg BW		
OVX-Control n = 17	340 ± 7	0.84 ± 0.02	2.48 ± 0.22	1.61 ± 0.04	3.72 ± 0.14	0.62 ± 0.02	18.4 ±1.9§
MCT n = 12	303 ±11*	1.08 ± 0.02*	3.64 ± 0.32*	2.02 ± 0.18*	7.02 ± 0.90*	0.62 ± 0.02	19.0 ± 1.0§
MCT+ Progesterone n = 11	310 ± 8*	1.00 ± 0.02*	3.27 ± 0.21*	1.77 ± 0.16	5.84 ± 0.72	0.63 ± 0.01	18.8 ± 1.0§
1F–ANOVA: p ≤	0.044	0.001	0.001	0.02	0.03	0.698	0.600

Табле 2

Effects of progesterone on monocrotaline-induced morphometric changes in pulmonary arterioles in ovariectomized rats.
*-p < 0.05 vs. OVX control; ** - p < 0.5, vs. MCT
Ефекти на прогестероне на монокроталински индуцирани морфометриски промени во белодробните артериоли кај овариектомизирани стаорци. *-p < 0.05 vs. OVX контроли; ** -p < 0.5, vs. MCT

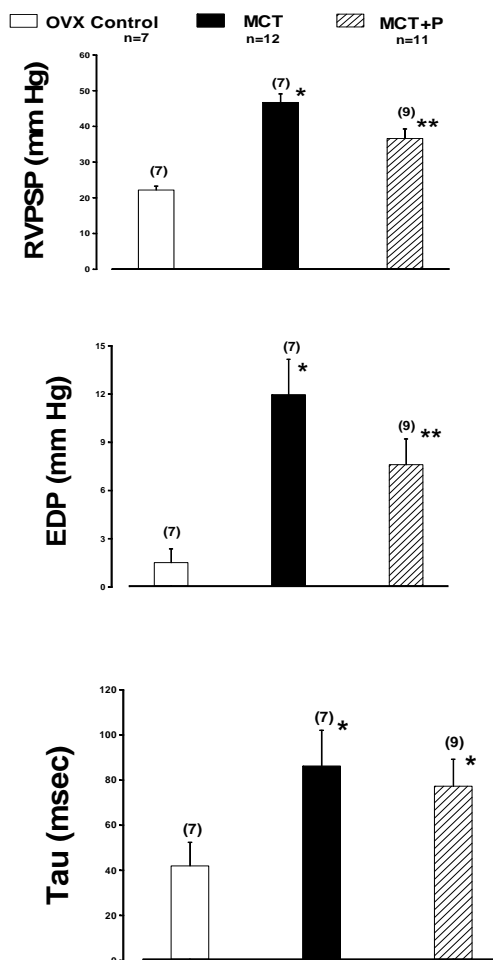| Group	Vessels Size (μ)	Diameter μ	Adventitia μ²	Wall μ²	Media μ²	Wall/Lumen Ratio	Media/Lumen Ratio
OVX-Control n = 7	59–248	123.7 ± 9.1	1356 ± 153	7138 ± 1154	5783 ± 1020	1.27 ± 0.09	1.00 ± 0.08
MCT n = 12	70–271	139.7 ± 7.9	2871 ± 352*	11945 ± 1522*	9073 ± 1272*	2.46 ± 0.26*	1.86 ± 0.23*
MCT+ Progesterone n = 11	73–242	134.2 ± 6.6	2442 ± 327*	8324 ± 767**	5882 ± 538**	1.43 ± 0.08**	1.05 ± 0.07**
1F-ANOVA: p ≤		0.387	0.005	0.02	0.04	0.001	0.001

Figure 1 – Right ventricular peak systolic pressure (RVPSP), right ventricular end diastolic pressure (EDP) and time constant of isovolumetric relaxation (Tau) in ovariectomized female rats (OVX-Control), in OVX animals with monocrotaline (MCT)-induced pulmonary hypertension, and diseased animals receiving progesterone (MCT + P). 1-F ANOVA, p < 0.001, * - p < 0.05 vs. OVX Cont, **-vs. OVX-Cont and MCT

Слика 1 – Десно вентрикуларен максимален систолен притисок (RVPSP), десно вентрикуларен краен дијастолен притисок (EDP) и временска константа на изоволуметрична релаксација (Тau) во овариектомизирани женски стаорци (OVX-Control), во OVX животни со монокроталин – индуцирана белодробна хипертензија (MCT), и заболени животни кои добиле прогестерон (MCT+P). 1-F ANOVA, p < 0.001, *-p < 0.05 vs. OVX Cont, **-vs. OVX-Cont и MCT
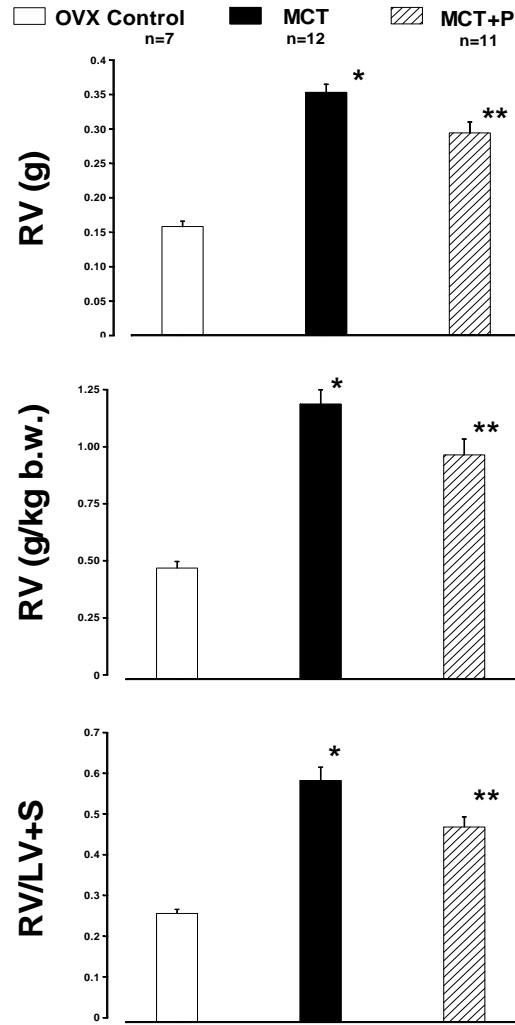
Figure 2 – Right ventricle (RV) free wall weight, RV per kg body weight and RV/left ventricle + septum ratio (RV/LV+S) in ovariectomized female rats (OVX-Control), OVX animals with MCT-induced pulmonary hypertension (OVX-MCT), and diseased animals receiving progesterone (OVX-MCT+P). 1-F ANOVA, p < 0.001, *- p < 0.05 vs. OVX Cont, **- vs. OVX Cont and OVX-MCT

Слика 2 – Тежина на десен вентрикуларен ѕид (RV), RV на кг телесна тежина и сооднос на RV/лева комора + septum (PV/LV+S) кај овариектомизирани женски стаорци (OVX-Control), OVX животни со MCT-индуцирана белодробна хипертензија (MCT), и заболени животни кои добиле прогестерон (OVX-MCT+P). 1-F ANOVA, p < 0.001, *- p < 0.05 vs. OVX Cont, **- vs. OVX Cont и OVX-MCT.
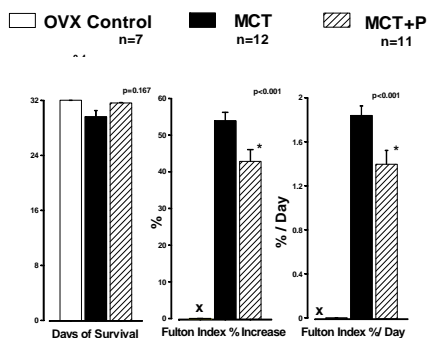
Figure 3 – Progression of right ventricular hypertrophy in ovariectomized animals with monocrotaline-induced pulmonary hypertension (MCT) and in diseased animals receiving progesterone (MCT+P); 1-F ANOVA, p < 0.001, *-p < 0.05 vs. OVX Cont; Expressed as absolute % increase in Fulton index (i.e., RV/LV+S ratio; Middle panel), and % increase in Fulton index per day (%/day; Left panel) – See methods section for explanation

Слика 3 – Прогресија на лево вентрикуларна хипертрофија во овариектомизирани животни со монокроталин-индуцирана белодробна хипертензија (MCT) и кај заболени животни кои добиле прогестерон (MCT + P); 1-F ANOVA, p<0.001, * - p < 0.05 vs. OVX Cont; Искажан како апсолутно зголемен % на Fulton-ов индекс (RV/LV+S сооднос; среден панел), и зголемен % на Fulton-ов индекс на ден (%/day; лев панел) – За објаснување види го делот методи
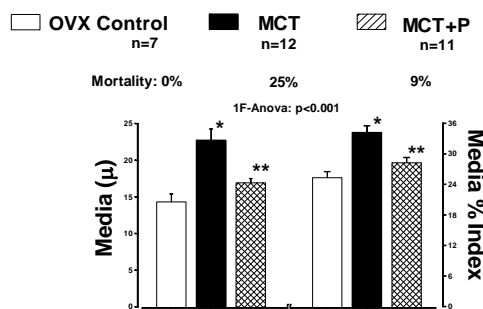

Figure 4 – Media thickens (left panel), and media % index (right panel) in ovariectomized female rats (OVX-Control), OVX rats with monocrotaline-induced pulmonary hypertension (MCT), and in diseased animals receiving progesterone (MCT+P). 1-F ANOVA, p < 0.001, * - p < 0.05 vs. OVX Cont, **- vs. OVX Cont and OVX-MCT

Слика 4 – Хипертрофија на медија (лев панел) и процент индекс на медија (десен панел) во белодробни артерии кај овариектомизирани женски стаорци (OVX-Control), OVX стаорци со монокроталинска белодробна хипертензија (MCT), и кај заболени животни кои добиваат прогестерон (MCT+P). 1-F ANOVA, p < 0.001, *-p < 0.05 vs. OVX Cont, **- vs. OVX Cont и OVX-MCT
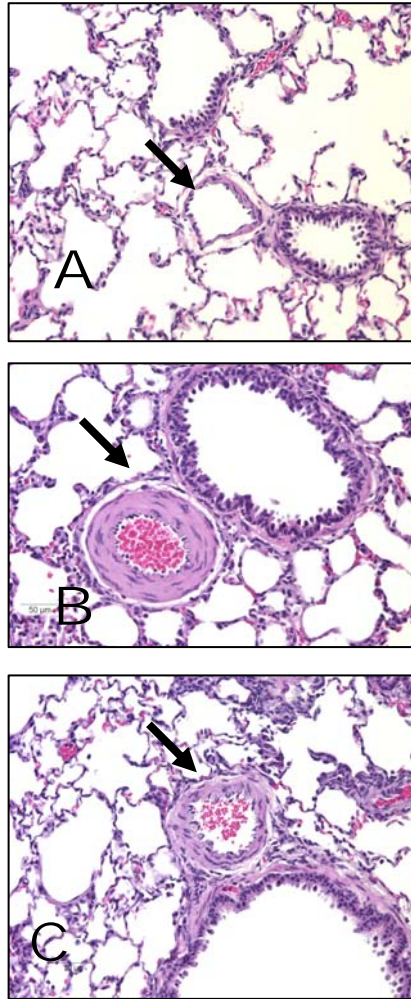
*Figure 5 – Pulmonary arteries in lungs from ovariectomized (OVX) control rat (**A**),
in OVX animal with monocrotaline (MCT) – induced pulmonary hypertension (**B**),
and in OVX-MCT rat receiving progesterone for 32 days (**C**)*
*Слика 5 – Белодробни артерии во бели дробови од овариектомизиран
контролен стаорец (**A**), во OVX животно со монокроталин (MCT) –
индуцирана белодробна хипертензија (**B**), и кај OVX-MCT стаорец кој
добива прогестерон 32 дена (**C**)*

Discussion

Administration of toxin monocrotaline (MCT) to rats produces a model
of pulmonary arterial hypertension. In this model, endothelial cell injury is an

early change (day 1–4) that precedes the thickening of the media of small pulmonary arteries (day 8–12) and the rise in pulmonary artery pressure that is noticeable by Day 12 after MCT administration [21, 22]. The endothelial damage is also followed by interstitial inflammation and focal alveolar edema [23]. Pulmonary arterial hypertension leads to right ventricular hypertrophy/failure and progressively increased mortality by Week Four [20, 21]. In order to induce pulmonary injury monocrotaline (MCT) needs to be metabolized in liver microsomes by cytochrome P-450 monooxygenase [24].

Bioactivation includes dehydrogenation of MCT to monocrotaline pyrrole, which when injected intravenously produces pneumotoxicity similar to that induced by MCT injection [25, 26]. Rats efficiently metabolize MCT with hepatic clearance of 29.2 μmol/h [27], and plasma half-life of approximately ~ 50 minutes [28]. After intravenous administration of C^{14}-MCT (60 mg/kg), in rats there is a rapid elimination of radioactivity with 90% recovery in urine and bile within a 7-hour period [28]. Therefore, to avoid possible interference of progesterone with MCT bioactivation, osmotic pumps delivering progesterone were implanted 8 hours after MCT administration. i.e. after biodeposition of MCT was completed.

In the present study, MCT induced pulmonary arterial hypertension, as evidenced by hypertrophy of small-size pulmonary arteries, increased RV/LV+S ratio and right ventricular pressure and produced high mortality (25%). Treatment with progesterone (at a dose that produces physiological plasma levels of this natural progestin) attenuated the development of monocrotaline (MCT)-induced pulmonary hypertension, lessened the right ventricular hypertrophy and failure, reduced vascular remodeling of small size pulmonary arteries, and decreased mortality in estrogen-deficient OVX rats. To the best of our knowledge, this is the first study to demonstrate the beneficial effects of progesterone in experimental pulmonary hypertension. Previously, others and we have demonstrated that ovariectomy exacerbates the MCT-induced pulmonary hypertension, while treatment with estradiol or its non-estrogenic metabolite 2-methoxyoestradiol eliminates the exacerbation of disease due to removal of ovaries. The important finding of the present study is that exacerbation of disease in MCT ovariectomized rats may be due, at least in part, to the lack of progesterone. Although the objective of this study was to study the effects of progesterone on development of pulmonary hypertension, the present data raises a question regarding the mechanism(s) of the protection of progesterone in pulmonary hypertension.

Dysfunctional endothelial cells, as during the early phase of pulmonary hypertension (including the MCT model), are characterized by impaired synthesis of vasoactive molecules, such as nitric oxide (NO), prostacyclin and endothelin. In this regard, treatment with NO donors l-arginine or molsidomine

[29, 30], overexpression of nitric oxide synthase or prostacyclin synthase [31, 32], and treatment with prostacyclin and NO or endothelin receptors antagonist [33–34] prevent or attenuate the development of experimental pulmonary hypertension in rats. Importantly, in contrast to its synthetic analogue medroxyprogesterone acetate, progesterone simulates synthesis of nitric oxide in isolated human endothelial cells and, *in vivo,* in aortas from OVX rat potentiates the effects of estradiol on NO synthesis [35, 36]. *In vitro,* similar to estradiol, progesterone, through endothelial formation of nitric oxide, induces rapid relaxation of pre-constricted vessels including the pulmonary artery [19, 37, 38]. The vascular relaxing response of progesterone is reduced by removal of endothelium and inhibition of NOS or guanylate cyclase [19]. Nonetheless, progesterone may also cause endothelium-independent vasorelaxation [39]. Furthermore, progesterone restores the endothelium-dependent vasodilatation in the mesenteric artery from OVX rats [40], and in OVX primates, the addition of progesterone results in less vascular injury than treatment with estradiol alone, suggesting that progesterone independently exerts vascular protective effects [41]. Also, in nitric oxide deficient pregnant or OVX female rats, progesterone potentiates the vasodilatory effects of calcitonin gene-related peptide [42]. At physiological concentrations, progesterone stimulates prostacyclin synthesis in human ECs [43] and inhibits serum and angiotensin-II stimulated synthesis and release of endothelin in human ECs [44], whereas its withdrawal results in increased endothelin release from microvascular endothelial cells [45]. Therefore, it is conceivable that progesterone may exhibit its protective effects in MCT-induce PH by favorably affecting the synthesis/bioavailability of NO, prostacyclin and/or endothelin.

The findings of this study suggest that exacerbation of disease in MCT ovariectomized rats may be due, at least in part, to the lack of progesterone. However, the limitation of this study is that experiments were conducted in estrogen-deficient animals and, therefore, it is not clear whether the same effects of progesterone on the development of disease would be seen in the presence of estradiol. The interaction between estradiol and progesterone is complex, not fully understood, and may be influenced by the type of vascular bed and the site of action [46, 47]. Progesterone was reported to potentiate or have no effect on the vasodilatory and vascular protective properties of E2, whereas synthetic progestins may be neutral or even antagonize the vascular effect of E2. Many of progesterone's biological effects are mediated by specific intracellular progesterone receptors (PR), which in different tissue are down-regulated by progesterone, but up-regulated by estradiol. The two PR isoforms (PR-A and PR- B) have been also identified in the lung of adult rats [48, 49]. The content of PR isoforms in the lung is lower in male rats than in female rats [48], and in female rats the expression of PR depends on the physiological levels of progesterone and estradiol. The highest content of PR isoforms in the

lungs of female rats is observed on the day of *proestrus* and the lowest on the day of *estrus*, suggesting that as in the other tissues, in the lung estradiol up-regulates and progesterone down-regulates progesterone receptors. It seems that up-regulation of PR by estradiol is mediated via ER-β [49]. It is not clear how this interaction between E2 and progesterone at receptor level would influence the effects of progesterone in pulmonary hypertension. The latter is of particular interest, since hormone replacement therapy, largely based on combined E2-progesterone administration, was suggested as a potential risk factor for pulmonary hypertension [50, 51]. Therefore, studies of E2 and progesterone interaction in pulmonary hypertension are warranted.

In summary, this study provides the first evidence that in ovariectomized female rats, progesterone has protective effects on the development of pulmonary hypertension. The presented data, and the fact that this study was conducted in estrogen-deficient animals, merit further examination of the effects of progesterone, including its interaction with estradiol, in the development of pulmonary hypertension.

REFERENCES

1. Rich S., Dantzker DR., Ayres SM., Bergofsky EH., Brundage BH., Detre KM., Fishman AP., Goldring RM., Groves BM., Koerner SK. (1987): Primary pulmonary hypertension: a national prospective study. *Ann Intern Med;* 107: 216–23.

2. Ghamra ZW. and Dweik RA. (2003): Primary pulmonary hypertension: An overview of epidemiology and pathogenesis. *Cleveland Clin J Med;* 70: S2–S8.

3. Burton RR., Besch EL. and Smith AH. (1968): Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. *Am J Physiol;* 214: 1438–1442.

4. McMurty IF., Firth CH., Will DH. (1973): Cardiopulmonary responses of male and female swine to stimulated high attitude. *J Appl Physiol;* 35: 459–462.

5. Rabinovitch M., Gamble WJ., Miettinen OS., Reid L. (1981): Age and sex influence on pulmonary hypertension of chronic hypoxia and recovery. *Am J Physiol Heart Circ Physiol;* 240: H62–H72.

6. Resta TC., Knaggy NL., Walker BR. (2001): Estradiol-induced attenuation of pulmonary hypertension is not associated with altered eNOS expression. *Am J Physiol Lung Cell Moll Physiol;* 280: L88–L97.

7. Farhat MY., Chen MF., Bhatti T., *et al.* (1993): Protection by oestradiol against the development of cardiovascular changes associated with monocrotaline pulmonary hypertension in rats. *Brit J Pharm;* 110: 719–23.

8. Tofovic SP., Zhang X., Jackson EK., Dacic S., Petrusevska G. (2006): 2-methoxyestradiol mediates the protective effects of estradiol in monocrotaline-induced pulmonary hypertension. *Vascular Pharmacology;* 45: 358–367.

9. Welter BH., Hansen EL., Saner KJ., Wei Y., Price TM. (2003): Membrane-bound Progesterone Receptor Expression in Human Aortic Endothelial Cells. *J Histochem Cytochem;* 51: 1049–1055.

10. Barberis MC., Veronese S., Bauer D., De Juli E., Harari S. (1995): Immunocytochemical detection of progesterone receptors. *A study in a patient with primary pulmonary hypertension Chest;* 107, 869–872.

11. Vazquez F., Rodriguez-Menzaneque JC., Lydon JP. *et al.* (1999): Progesterone regulates proliferation of endothelial cells. *Biol Chem;* 274: 2185–2192.

12. Ingegno MD., Money SR, Thelmo W, Greene GL, Davidian M., Jaffe BM. Pertschuk LP. (1988): Progesterone receptors in the human heart and great vessels. *Lab Invest;* 59: 353–256.

13. Perrot-Applanat M., Groyer-Picard MT., Garcia E., Lorenzo F., Milgrom E. (1988): Immunocytochemical demonstration of estrogen and progesterone receptors in muscle cells of uterine arteries in rabbits and humans. *Endocrinology;* 123: 1511–1519.

14. Nakamura Y., Suzuki T., Inoue T. *et al.* (2005): Progesterone receptor subtypes in vascular smooth muscle cells of human aorta. *Endocrine J;* 52: 245–252.

15. Morey AK., Pedram A., Razandi M., Prins BA., Hu RM., Biesiada E., Levin ER. (1997): Estrogen and progesterone inhibit vascular smooth muscle proliferation. *Endocrinology;* 138: 3300–3339.

16. Lee WS., Harder JA., Yoshizumi M., Lee ME., Haber E. (1997): Progesterone inhibits arterial smooth muscle cell proliferation. *Nat Med;* 3: 1005–1008.

17. Lee W-S., Lliu C-W., Juan S-H., Liang Y-C., Ho P-Y., Lee Y-H.. (2003): Molecular mechanism of progesterone-induced antiproliferation in rat aortic smooth muscle cells. *Endocrinology;* 144: 2785–2790.

18. English KM., Jones RD., Jones TH., Morice AH., Channer KS. (2001): Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Hormone Metab Res;* 33: 645–52.

19. Li H-F., Zhen T-Z., Li W., Qu S-Y., Zhang C-L. (2001): Effects of progesterone on the contractile response of isolated pulmonary artery in rabbits. *Can J Physiol Pharmacol/ Rev Can Physiol Pharmacol;* 79: 545–55-.

20. Fulton RM., Hutchinson EC., Morgan-Jones A. (1952): Ventricular weight in cardiac hypertrophy. *Br Heart J;* 14: 413–420.

21. Rosenberg H., Rabinovitch M. (1988): Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. *Am J Physiol;* 255: H1484–H1491.

22. Cowan KN., Heilbut A., Humpl T., Lam C., Ito S., Rabinovitch M. (2000): Complete reversal of fatal pulmonary hypertension in rat by a serine elastase inhibitor. Nature Medicine 6: 698–701.

23. Wilson DW., Segal HJ., Pan IC. *et al.* (1992): Mechanisms of pathology of monocrotaline toxicity. *Crit Rev Toxicol;* 22: 307–325.

24. Mattocks AR., White INH. (1970): Estimation of metabolites of pyrrolizidine alkaloids in animal tissues. *Anal Biochem;* 38: 529–35.

25. Schultze AE., Wagner JG., White SM., Roth RA. (1991): Early indications of monocrotaline pyrrole-induced lung injury in rats. *Toxicol Appl Pharmacol;* 109: 41–50.

26. Hoorn CM., Roth R. (1992): Monocrotaline pyrrole alters DNA, Rm and pulmonary toxicity of monocrotaline using isolated perfused liver and lung. *Biochem Pharmacol;* 33: 2485–91.

27. Yan CC., Cooper RA., Huxtable RJ. (1995): The comparative metabolism of the four pyrrolizidine alkaloids, seneciphylline, retrorsine, monocrotaline, and trichodesmine in the isolated, perfused rat liver. *Toxicol Appl Pharmacol;* 133: 277–284,

28. Estep JE., Lame WM., Morin D. *et al.* (1991): [^{14}C] Monocrotaline kinetics and metabolism in the rat. *Drugs Met Disp;* 19: 135–139.

29. Mitani Y., Maruyama K., Sakurai M. (1997): Prolonged administration of L-arginine ameliorates chronic pulmonary hypertension and pulmonary vascular remodeling in rats. *Circulation;* 96: 689–97.

30. Mathew Rajamma, Elizabeth S., Gloster T. *et al.* (1997): Role of inhibition of nitric oxide production in monocrotaline-induced pulmonary hypertension. *J. Appl. Physiol;* 82(5): 1493–1498.

31. Campbell AI., Kuliszewski MA., Stewart DJ. (1999): Cell-based gene transfer to the pulmonary vasculature: Endothelial nitric oxide synthase overexpression inhibits monocrotaline-induced pulmonary hypertension. *Am J Res Cell & Mol Biol;* 21: 5675–75.

32. Nagaya N., Yokoyama C., Kyotani S. *et al.* (2000): Gene transfer of human prostacyclin synthase ameliorates monocrotaline-induced pulmonary hypertension in rats. *Circulation;* 102: 2005–2010.

33. Ueno M., Miyauchi T., Sakai S., Goto K., Yamaguchi I. (2000): Endothelin-A-receptor antagonist and oral prostacyclin analog are comparably effective in ameliorating pulmonary hypertension and right ventricular hypertrophy in rats. *J Cardiovasc Pharm;* 36(5 Suppl 1): S305–310.

34. Hill LL., Pearl RG. (1999): Combined inhaled nitric oxide and inhaled prostacyclin during experimental chronic pulmonary hypertension. *J Appl Physiol;* 86: 1160–1164.

35. Karas RH., van Eickels M., Lydon JP., Roddy S., Kwoun M., Aronovitz M., Baur WE., Conneely O., O'Malley BW., Mendelsohn ME. (2001): Complex role for the progesterone receptor in the response to vascular injury *J Clin Invest;* 108: 611–618.

36. Simoncini T., Mannella P., Fornari L., Caruso A., Willis MY., Garibaldi S., Baldacci C., Genazzani AR. (2004): Differential signal transduction of progesterone and medroxyprogesterone acetate in human endothelial cells. *Endocrinology;* 145: 5745–5756.

37. Zhang M., Wang GJ., Benishin CG., Pang PK. (2002): Rapid effects of progesterone on the contraction of rat aorta in-vitro. *J Pharmacy & Pharmacol;* 54: 1529–1534.

38. Orshal JM., Khalil RA. (2004): Gender, sex hormone, and vascular tone. *J Am Physiol REgul Integr Comp Physiol*; 286: R233–R249.

39. Jiang C., Sarrel PM., Lindsay DC., Poole-Wilson PA., Collin P. (1992): Progesterone induces endothelium-independent relaxation of rabbit coronary artery in vitro. *Eur J Pharmacol:* 211: 163–167.

40. Chataigneau T., Zern M., Chataigneau M., Hudlett F., Hirn C., Pernot F., Schini-Kerth VB.: Chronic treatment with progesterone, but not medroxyprogesterone acetate, restores the endothelial control of vascular tone in the mesenteric artery of ovariectomized rats. *Menaupose;* 11: 255–263, 204.

41. Kushwaha RS., Lewis DS., Carey KD., McGill HC Jr. (1999): Effects of estrogen and progesterone on plasma lipoproteins and experimental atherosclerosis in the baboon (papio sp). *Arterioscler Thromb;* 11: 23–31.

42. Gangula P., wimalawansa SJ., Yallampalli C. (1997): Progesterone up-regulates vasodilator effects of calcitonin gene-related peptide in N sup-G-nitro-L-arginine methyl ester-induced hypertension. *Am J Obst Gynecol;* 176: 894–900.

43. Hermenegildo C., Oviedo PJ., Garcia-Martinez MC., Garcia-Perez MA., Tarin JJ., Cano A. (2005): Progestins stimulate prostacyclin production by human endothelial cells. *Human Reproduction;* 28: 1554–1566.

44. Morey AK., Razand M., Pedram A., Hu R-M., Prins BA., Levin ER. (1998): Oestrogen and progesterone inhibit the stimulated production of endothelin-1. *Biochem J;* 338: 1097–1105.

45. Edlund M., Andersson E., Fried G. (2004): Progesterone withdrawal causes endothelin release from cultured human uterine microvascular endothelial cells. *Human Reproduction;* 19: 1272–1280.

46. Mabry MM., Zamudio S., Stevens T. *et al.* (1995): Estrogen, progesterone, and vascular reactivity: Potential cellular mechanism. *Endocrine Rev;* 16: 739–751.

47. Thompson J., Khalil R. (2003): Gender differences in the regulation of vascular tone. *Clin and Exp Pharmacol and Physiol;* 30: 1–15.

48. Gonzales-Arenas A., Neri-Gomez T., Guerra-Araiza C., Camacho-Arroyo I. (2004): Sexual dimorphism in the content of progesterone and estrogen receptors, and their cofactors in the lung of adult rats. *Steroids;* 69: 351–356.

49. Gonzales-Arenas A., Villmar-Cruz O., Guerra-Araiza C., Camacho-arroyo I. (2003): Regulation of progesterone receptor isoforms expression by sex steroids in the lung. *J Steroid Biochem Mol Biol;* 85: 25–31.

50. Morse JH., Horn EM., Barts RJ. (1999): Hormone replacement therapy. A possible risk factor in carriers of familial primary pulmonary hypertension. *Chest;* 116: 847.

51. Kleiger RE., Boxer M., Ingham RE., Harrison DC. (1976): Pulmonary hypertension in patients using oral contraceptives. A report of six cases. *Chest;* 69: 143–147.

Р е з и м е

ПРОГЕСТЕРОНОТ ГИ ИНХИБИРА ВАСКУЛАРНИТЕ ОШТЕТУВАЊА И ЈА НАМАЛУВА МОНОКРОТАЛИН-ИНДУЦИРАНАТА БЕЛОДРОБНА ХИПЕРТЕНЗИЈА КАЈ ЕСТРОГЕН ДЕФИЦИТНИ СТАОРЦИ

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А п с т р а к т: Белодробната артериска хипертензија (ВХ) е предоминантна болест кај младите жени. Сè уште малку се знае за ефектите на женските полови хормони на БХ. Женските стаорци развиваат помалку силна БХ споредено со машките стаорци, и овариектомијата (ОВК) ја влошува БХ. Иако ОВК-стаорците третирани со естрадиол развиваат помалку силна болест, улогата на прогестеронот кај ОВК-индуцираното влошување на болеста досега не е испитувано. Познато е дека прогестеронот ги шири крвните садови и ја инхибира пролиферацијата на ендотелните и мазномускулните клетки. Од тие причини, нашата хипотеза беше дека прогестеронот може да придонесува за заштитните ефекти во експериментална БХ. Вкупно 30 женски стаорци беа овариектомизирани и ОВК-стаорците беа третирани по случаен избор или со физиолошки раствор (ОВК – контролна група, монокроталин (60 мг/кг и.п.; ОВК–МКТ група; н = 12), или МКТ плус прогестерон (30 мг/кг/х преку осмотски минипумпи; ОВК–МКТ + П група; н = 11). Животните беа оперирани по 32 дена за in situ (отворен граден кош) мерења на врвниот систолен притисок на десната комора (ДВ), (ДВСП) и крајните дијастолни притисоци (ДВКДП), а ткивни примероци беа добиени за морфометриска и хистолошка анализа. Администрацијата на МКТ го покачува ДВСП (22,2 ± 1,1 vs. 46,7 ± 2,4 mmHG) и ДВКДП (1,51 ± 0,86 vs. 11,9 ± 2,2 mmHg,), го зголемува соодносот ДВ/лева комора + преграда (0,256 ± 0,010 vs. 0,582 ± 0,033, ОВК vs. ОВК–МКТ) и индуцира медијална хипертрофија на

белодробните артерии со мал калибар. Кај овариектомизирани белодробно-хипертензивни стаорци, третманот со прогестерон ја намалува тежината на болеста (група на ОВК–МКТ + П: ДВСП = 36,6 ± 2,3 mmHg; ДВ/ЛВ + П = 0,468 ± 0,025; ДВКДП = 7,5 + 1,5 mmHg) и го намалува морталитетот (9% vs. 25%; ОВК–МКТ + П vs. ОВК–МКТ).

Оваа студија овозможува прва потврда дека кај естроген дефицитни стаорци, прогестеронот има заштитни ефекти кај МКТ-индуцирана БХ. Потребна е натамошна евалуација на улогата на прогестеронот и неговата интеракција со естрогени кај белодробна хипертензија.

Клучни зборови: белодробна хипертензија, прогестерон, естрогени хормони, васкуларни оштетувања.

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