Estradiol Metabolites Attenuate Monocrotaline-Induced Pulmonary Hypertension in Rats

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Abstract: Pulmonary arterial hypertension (PH) is a deadly disease characterized by pulmonary arterial vasoconstriction and hypertension, pulmonary vasculature remodeling, and right ventricular hypertrophy. Our previous in vivo studies, performed in several models of cardiac, vascular, and/or renal injury, suggest that the metabolites of 17β-estradiol may inhibit vascular and cardiac remodeling. The goal of this study was to determine whether 2-methoxyestradiol (2ME), major non-estrogenic estradiol metabolite, prevents the development and/or retards the progression of monocrotaline (MCT)-induced PH. First, a total of 27 male Sprague Dawley rats were injected with distillated water (Cont, n = 6) or monocrotaline (MCT; 60 mg/kg, i.p.; n = 21). Subsets of MCT animals (n = 7 per group) received 2ME or its metabolic precursor 2-hydroxyestradiol (2HE; 10 μg/kg/h via osmotic minipumps) for 21 days. Next, an additional set (n = 24) of control and MCT rats was monitored for 28 days, before right ventricular peak systolic pressure (RVPSP) was measured. Some pulmonary hypertensive animals (n = 8) were treated with 2ME (10 μg/kg/h) beginning from day 14 after MCT administration. MCT caused pulmonary hypertension (ie, increased right ventricle/left ventricle + septum [RV/LV+S] ratio and wall thickness of small-sized pulmonary arteries, and elevated RVPSP) and produced high and late (days 22 to 27) mortality. Pulmonary hypertension was associated with strong proliferative response (PCNA staining) and marked inflammation (ED1 + cells) in lungs. Both metabolites significantly attenuated the RV/LV+S ratio and pulmonary arteries media hypertrophy and reduced proliferative and inflammatory responses in the lungs. Furthermore, in diseased animals, 2ME (given from day 14 to 28) significantly decreased RVPSP, RV/LV+S ratio and wall thickness, and reduced mortality by 80% (mortality rate: 62.5% vs. 12.5%, MCT vs. MCT+2ME day 14 to 28). This study provides the first evidence that 2ME, a major non-estrogenic, non-carcinogenic metabolite of estradiol, prevents the development and retards the progression of monocrotaline-induced pulmonary hypertension. Further evaluation of 2ME for management of pulmonary arterial hypertension is warranted.

Key Words: 17β Estradiol, metabolites, pulmonary arterial hypertension

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Pulmonary arterial hypertension (PH) is severe disease characterized by progressive increase in pulmonary arterial pressure that ultimately leads to right heart failure and death. Although initially it was considered to be a disease of abnormal pulmonary vasoconstriction[1,2] recently vascular remodeling has been recognized as an important component in the pathogenesis of this deadly disease.[3,4] In this regard, as the disease advances, the abnormal vasoconstriction became less important and vascular remodeling increasingly more important.[5] Furthermore, impaired angiogenesis with tumor-like (monoclonal) proliferation of apoptosis-resistant endothelial cells was suggested as a unique/underlining process in severe pulmonary hypertension.[6] Therefore, the anti-proliferative/anti-angiogenic agents that target not only pulmonary vascular (medial) remodeling, but also endothelial remodeling may be effective in the management of this disease.

Estradiol is protective in experimental models of cardiovascular disease. This is not surprising taking into account the anti-mitogenic effects of estradiol on vascular smooth muscle cells (VSMC[7–9]) and its favorable effects on vasoactive and growth regulating factors, such as nitric oxide (NO), prostacyclin, and endothelin.[10–12] Similarly, in different species, it has been reported that female animals developed less severe PH and that estradiol may be protective in pulmonary hypertensive animals.[13–16] However, pulmonary arterial hypertension is a disease with apparent worldwide female preponderance[17] and estrogenic effects (ie, trombogenic effects of estradiol) are unwelcome in patients with pulmonary arterial hypertension.

There is a line of evidence suggesting that several of the cellular effects of 17β-estradiol (E2) are largely mediated by its downstream metabolites.[18] The major metabolic pathway for estradiol includes C2 hydroxylation that leads to the formation of 2-hydroxyestradiol (2-HE). This metabolite has little estrogenic activity[19] and is quickly (t/2 = 90')[20,21] converted (by COMT) to 2-methoxyestardiol (2ME), a major estradiol metabolite with no estrogenic activity. 2-Methoxyestradiol has a 500- and 3500-times lower affinity than estradiol for estrogen receptor (ER) α and ER(β, respectively, whereas 2HE retains some binding activity.[19] Furthermore, the effects of 2ME in ER-positive and ER-negative tumor cells and in cardiovascular cells (including endothelial and vascular smooth muscle cells) are not blocked by specific estrogen

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receptor antagonists.[18,22–24] Estradiol metabolites, once held to be inactive, are even more potent anti-mitogens than estradiol itself.[22,25–27] Moreover, 2-ME is more potent than estradiol in increasing NO and prostacyclin synthesis and in inhibiting the endothelin synthesis and MAPK activity in endothelial cells.[28–30] The fact that the order of potency for these effects (2ME > 2HE > E2) is opposite to the order of potency binding to or activation of estrogen receptors, also suggests the involvement of estrogen receptor-independent mechanisms.

In contrast to the similar effects on other cardiovascular cells, estradiol and 2ME have divergent effects on endothelial cells (ECs). That is, E2 is mitogenic, pro-angiogenic, and anti-apoptotic, whereas 2ME is anti-mitogenic, anti-angiogenic, and pro-apoptotic in endothelial cells. This may have significant ramifications in severe PH that involves EC growth that is similar with neoplastic cell growth (supra vide). Importantly, the anti-angioproliferative/apoptotic approach used for management of cancer has been recently proposed for management of severe PH[31] and, in early clinical trials, 2ME was safely and successfully tested in cancer patients.[22]

Our previous studies, performed in several different models of cardiovascular and/or renal injury,[32–36] also suggest that estradiol metabolites have strong antiproliferative effects and may inhibit vascular and cardiac remodeling in vivo. Therefore, we hypothesized that by inhibiting vascular remodeling, estradiol metabolites may confer protection in pulmonary hypertension. The goal of this study was to determine whether 2-methoxyestradiol, major non-estrogenic and non-carcinogenic metabolite of estradiol and its metabolic precursor 2-hydroxyestradiol, prevent the development and/or retard the progression of monocrotaline (MCT)-induced pulmonary hypertension in rats.

MATERIALS AND METHODS

Animals

All experiments were carried out in accordance with the institutional guidelines for animal welfare, and the Animal Care and Use Committee approved experimental protocols. A total of 51 male, 12-week-old Sprague Dawley rats were used. Animals were housed in the University of Pittsburgh Medical Center animal care facility (temperature 22°C; light cycle, 12 hours; relative humidity, 55%). Rats were fed Pro Laboratory RHM 3000 rodent diet (PMI Nutrition, Inc, St Louis, MO) and were given water ad libitum.

Protocol I: Effects of Estradiol Metabolites on Development of Pulmonary Arterial Hypertension

Twenty-seven male Sprague Dawley rats were randomly assigned to receive by intraperitoneal injection either mixture of 1 mL 1N HCl neutralized with 1.0 N NaoH and diluted with distillated water (10 mL/kg, Control group; n = 6) or monocrotaline (MCT, 60 mg/kg, n = 21). Monocrotaline (Sigma, St Louis, MO) was dissolved in 1N HCl at a concentration of 100 mg/mL, neutralized with 1 N NaOH, and diluted with distillated water to 6 mg/mL. Six hours after the MCT administration control animals and a subset of MCT rats (n = 7) were implanted with osmotic minipumps (model 2ML4, Altza, Palo Alto, CA) delivering vehicle (polyethylene glycol 400, 2.5 μl/h; Control and MCT groups, respectively). Subsets of MCT-treated animals were implanted with osmotic minipumps delivering either 2-hydroxyestradiol (10 μg/kg/h, MCT+2HE group, n = 7) or 2-methoxyestradiol (10 μg/kg/h, MCT+2ME group, n = 7). Assignment to the MCT, MCT+2HE, and MCT+2ME groups was conducted randomly.

After 21 days of treatment, each animal was anesthetized with pentobarbital (45 mg/kg) and instrumented for measurements of blood pressure and heart rate as described previously.[32,33] Animals were euthanized by anesthetic overdose and heart, lungs. and main pulmonary artery 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

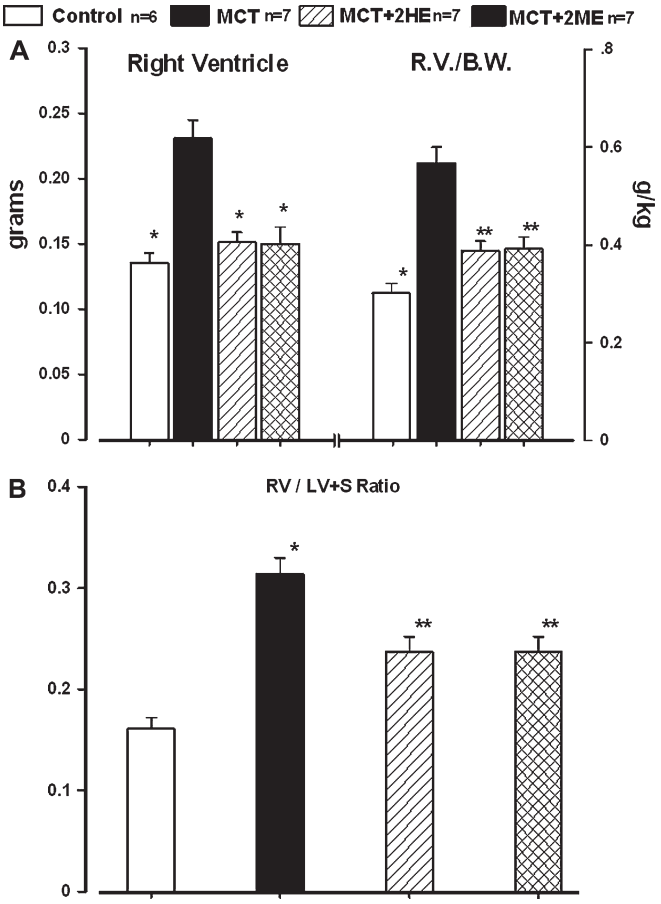

FIGURE 1. Right ventricle free wall wet weight (A) and right ventricle (RV) left ventricle plus septum (LV+S) ratio (B) in control animals, in rats with monocrotaline-induced pulmonary hypertension (MCT), and in MCT rats treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME). 1-F Anova, $P < 0.001$; Fisher LSD test for post-hoc comparison: *$P < 0.05$ vs. Control; **$P < 0.05$ vs. Control and MCT groups.

ratio (RV/LV+S). The right ventricle, left ventricle, main pulmonary artery, and the right inferior lobes of the lungs were cut and fixed in 10% buffered formalin, embedded in paraffin, and cut at 4-μm sections for light microscopy and immunohistochemical staining.

To assess cell proliferation, tissue sections (4 μm) were incubated with a mouse monoclonal anti-proliferating cell nuclear antigen (PCNA) at 1/200 dilution (Dako co, Carpenteria, CA). This antibody shows positive nuclear staining of proliferating cells. The number of interstitial monocytes/macrophages was studied using a polyclonal anti-ED1 antibody (Serotec, Raleigh, NC). This antibody specifically stains positive the cytoplasm of alveolar and interstitial macrophages. Nonspecific staining was assessed by replacing the primary antibody with affinity-purified, nonimmune, rabbit IgG (R&D Systems). Sections were washed and developed further according to the directions of the manufacturer (Dako, Carpinteria, CA) using an LSAB2 kit, which contained second antibody linked to avidin and peroxidase-conjugated to biotin. The area and intensity of positive immunohistochemical staining for PCNA was assessed quantitatively with a SAMBA 4000 image analyzer (Image Products International, Chantilly, VA) using specialized computer software (Immuno-Analysis, version 1.4; Microsoft, Richmond, WA), a color video camera, and a Compaq computer. The image analyzer is an integrated system of Windows-based software (Microsoft, Redmond, VA) for densitometric, morphometric, and red-green-blue to hue-saturation-intensity colorimetric analysis of cells and tissues. Software designed for immunostaining analysis enabled the operator to set a density threshold value by averaging several fields on the negative control tissues in which the primary antibody was replaced by isotypic control IgG. Background subtraction then was performed automatically on every tissue. The slides were examined at 400× magnification. Ten high-power fields per section were selected randomly and assessed for the intensity of staining, vascular wall thickening, and ED1 positively stained macrophages. The results for PCNA are reported as labeling index, which represents the percentage of total area examined that stained positively. Staining intensity of positive areas (mean optical density) also was assessed by quantitative image analysis. A quick score then was calculated for each section examined (labeling index × mean optical density). Quick scores and labeling indexes gave analogous results with respect to differences observed among the study groups. The inflammatory response data are expressed as the total number of ED1-positive cells per 10 microscopic high-power fields. To assess pulmonary vascular remodeling, pulmonary arterial wall smooth muscle cells were stained using a mouse monoclonal and anti-smooth muscle-alpha actin antibody at a dilution of 1/100 (Laboratory Vision, Fremont, CA). The vessel walls and media thickening were measured in the peripheral lung fields at approximately equal distances from the pleural lining, using morphometric methods using a digital planimeter on the SAMBA 4000 image analyzer. Only vessels with an approximately circular profile (cross-sectional cuts) were studied.

Protocol II: Effects of 2-Methoxyestradiol on Progression of Pulmonary Arterial Hypertension

A total of 24 male Sprague Dawley rats used to determine whether in animals with MCT-induced pulmonary hypertension, administration of 2-ME retards the progression

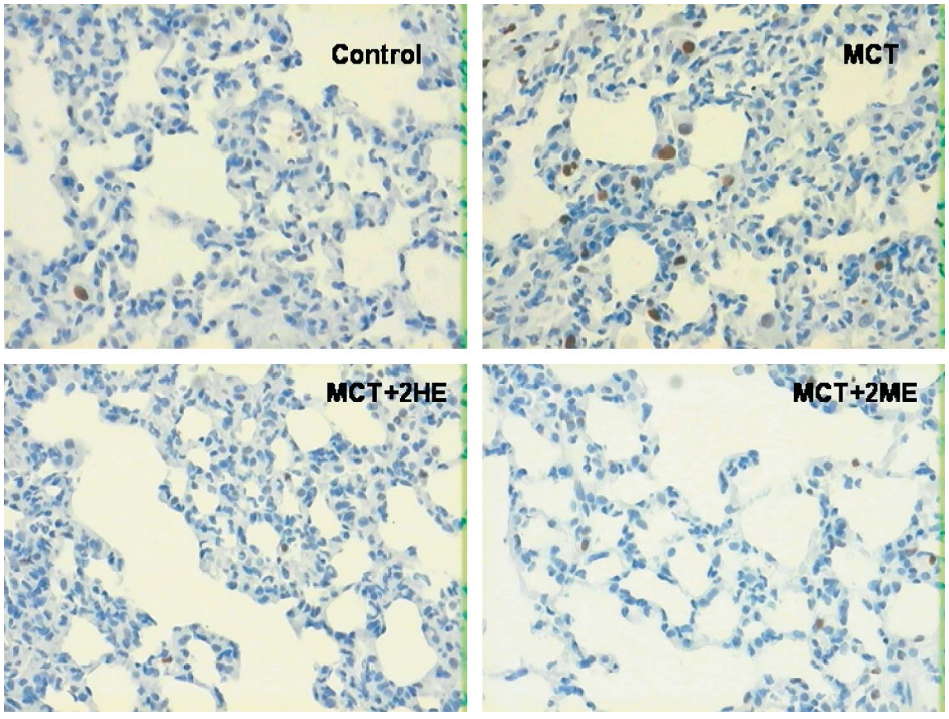

FIGURE 2. Representative immunohistochemistry for proliferating cells (PCNA staining) in lungs from control animal, rat with monocrotaline-induced pulmonary hypertension (MCT), and from MCT rats treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME).

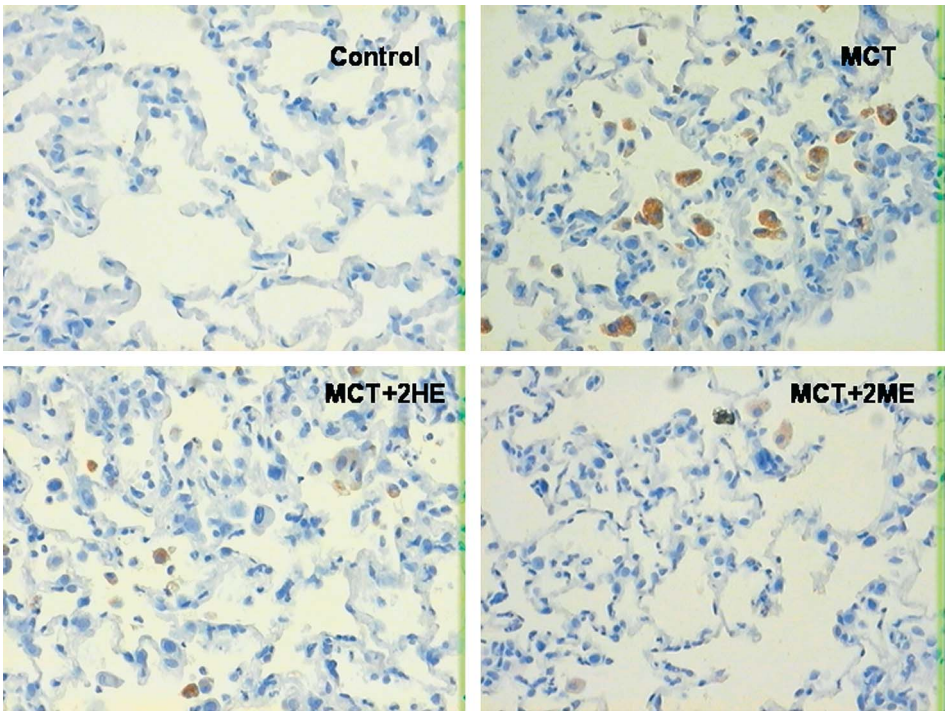

FIGURE 3. Representative immunohistochemistry of inflammatory cells (ED1+ cells) in the lungs from control animal, rat with monocrotaline-induced pulmonary hypertension (MCT), and MCT rats treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME).

of disease and reduces mortality. Animals were randomly assigned to receive by intraperitoneal injection either vehicle (Cont; n = 8) or MCT 60 mg/kg (n = 16), as described in Protocol I. Two weeks later some of the MCT rats were implanted osmotic minipumps delivering 2ME at rate of 10 μg/kg/h (MCT+2ME 14–28-day group; n = 8). Because in this model mortality begins rapidly to increase 3 weeks after MCT administration,[37–39] we monitored the animals for 4 weeks, before acute measurements of right ventricular pressure and morphometric analyses were conducted. Twenty-eight days after administration of MCT, surviving animals were anesthetized and instrumented for measurement of right ventricular peak systolic pressure (RVPSP). Briefly, a PE-240 polyethylene catheter was inserted into the trachea to facilitate breathing. 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 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 30-minute stabilization period, RVPSP was recorded over 20 minutes. Animals were euthanized by anesthetic overdose and heart and lungs were taken for morphometric analyses as described in Protocol I. Also, to monitor for any estrogenic activity, testicles and seminal vesicle were removed and their weight measured.

Statistical analyses were performed using the Number Cruncher Statistical software program (Kaysville, UT). Group comparisons were performed by a one-factor analysis of variance (1-F ANOVA), followed by post-hoc comparison using the Fisher LSD test. The probability value of $P < 0.05$ was considered statistically significant. All data are presented as mean ± SEM.

RESULTS

In animals monitored for 21 days (Protocol I) no changes in systemic blood pressure were detected in MCT rats compared with control animals, and estradiol metabolites had no effect on blood pressure (data not shown). Monocrotaline-treated animals had reduced body weight compared with control animals with intact lungs (450 ± 9, 401 ± 8, 397 ± 15, and 389 ± 11 g, CONT, MCT, MCT+2HE and MCT+2ME group, respectively) and MCT increased heart and lung wet weights (data not shown). More importantly, MCT induced right ventricular hypertrophy, as evidenced by a 70% increase in right ventricle free wall weight (Fig. 1A) and almost doubled RV/LV+S ratio, a specific index of RV hypertrophy (Fig. 1B). Continuous 21-day microinfusion of estradiol metabolites attenuated the MCT-induced increase in heart and lungs weights and reduced MCT-induced right ventricular hypertrophy (Figs. 1A and 1B).

The representative immunohistochemistry for proliferating cells in the lungs are presented in Figure 2 and quantitative analysis of proliferative response (PCNA staining) in the lungs is presented in Figure 4A. Monocrotaline-induced endothelial damage and subsequent pulmonary arterial hypertension were accompanied by marked proliferative response. Increased staining for proliferating cells was detected in the lungs in MTC-treated animals as compared with control animals. Increased presence of PCNA-positive cells in the right ventricle suggested increased proliferation most likely of cardiac fibroblasts whereas, in the pulmonary artery, increased

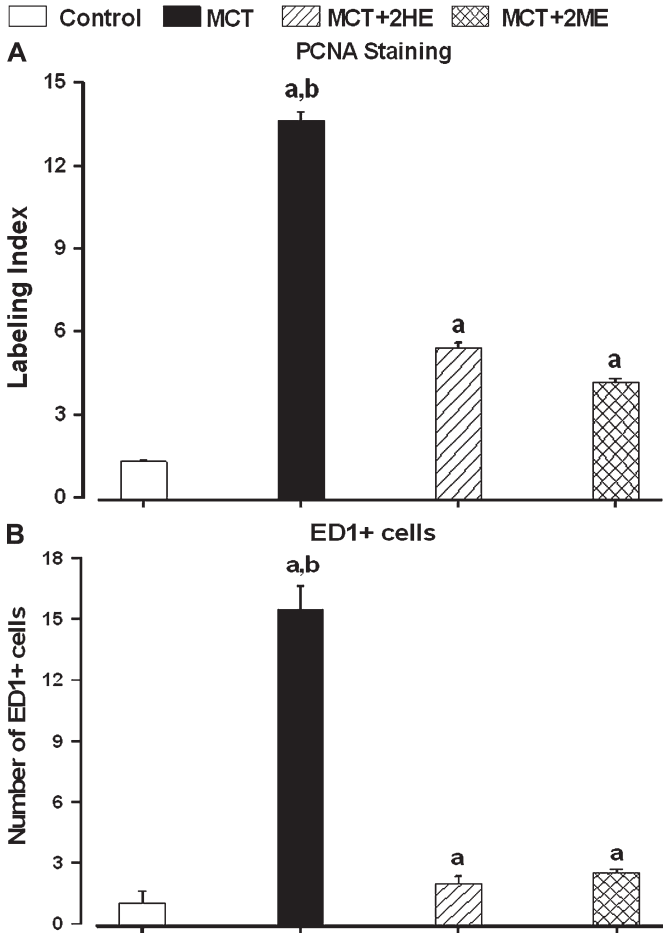

FIGURE 4. Proliferating cells (PCNA staining) and inflammatory cells (ED1+ cells) in lung, in control animals, in rats with monocrotaline-induced pulmonary hypertension (MCT), and in MCT rats treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME). 1F-Anova, $P < 0.05$; Fisher LSD test for post-hoc comparison: (a) $P < 0.05$ vs. Control; (b) $P < 0.05$ vs. MCT+2HE and MCT+2ME groups.

number of PCNA-positive cells in the media and adventitia (data not shown), suggested an increased proliferation of VSMCs and adventitial fibroblast. Both 2HE and 2ME significantly inhibited the proliferative response in the lungs (−60% and −70%, 2HE and 2ME, respectively; Fig. 4A). The development of pulmonary arterial hypertension was also associated with significant inflammation in the lungs (Figs. 3 and 4B). Estradiol metabolites almost completely abolished the inflammatory response in the lungs (Fig. 3). The development of pulmonary hypertension in MCT-treated animals was associated with marked vascular remodeling. Immunohistochemical analysis of small-sized pulmonary arteries (smooth muscle α-actin staining) detected marked media hypertrophy in MCT rats (increased wall thickness by 87% in MCT groups compared with control animals) (Figs. 5 and 6). Importantly, both estradiol metabolites attenuated MCT-induced hypertrophy of the media (−50% and −40%, MCT+2HE and MCT+2ME, respectively).

In the second set of experiments, animals with MCT-induced PH were monitored for 28 days. Pulmonary hypertensive animals had reduced body weight as compared with control animals, and 2ME did not alter body weight in MCT rats (data not shown). Single injection of monocrotaline induced pulmonary hypertension; MCT animals had markedly elevated right ventricle peak systolic pressure (RVPSP Fig. 7, left panel), augmented right ventricle free wall weight (228 ± 8 vs. 546 ± 15 mg, Control vs. MCT) and RV/LV+S ratio (Fig. 7B, right panel), and MCT produced vascular remodeling (Fig. 7, middle panel). Pulmonary and cardiac changes resulted with late (day 22–27) but high (62.5%) mortality. Treatment with 2ME from day 14 to 28 after induction of pulmonary hypertension retarded the progression of disease. Animals in MCT+2ME day14–28 group had lower right ventricle weight (546 ± 15 vs. 437 ± 30 mg, MCT vs. MCT+2ME 14–28 days), and reduced RVPSP and RV/LV+S ratio (Fig. 7). Importantly, 2ME significantly reduced mortality (12.5 vs. 62.5%; MCT+2ME day 14–28 group vs. MCT group; $P < 0.001$). The beneficial effects in male animals occurred in the absence of estrogenic effects. Treatment with 2ME for 14 days did not alter the weight of testicles and seminal vesicle.

DISCUSSION

In the present study, in male rats MCT induced pulmonary arterial hypertension and histopathological changes in the lungs similar to those reported previously.[37–39] Single injections of MCT resulted in vascular remodeling of small-sized pulmonary arteries, increased RV/LV+S ratio and right ventricular pressure, and high mortality. Importantly, estradiol metabolites attenuated the development of MCT-induced pulmonary hypertension. The significant (>50%) reduction in vascular remodeling and right ventricular hypertrophy was associated with strong anti-proliferative and anti-inflammatory effects in the lungs. Moreover, in animals with fully developed disease, treatment with 2ME retarded the progression of disease and reduced mortality by 80%. 2-Methoxyestradiol exhibited no estrogenic effects (ie, no effects on weight of testicles and seminal vesicles).

The protective effects of 2-ME were attained by a dose (10 μg/kg/h), which was shown to produce plasma concentrations of 3000 to 6000 pg/mL of 2ME (10–20 nmol/L; 20). Although these concentrations are 20 to 100 times higher than the reported concentrations of 2ME or estradiol in pre- and post-menopausal woman and in man,[40,41] they are considered "high physiological" concentrations because they are as high as those seen in newborns and women during the last trimester of pregnancy.[40] The beneficial effects of 2ME in male rats with MCT-induced pulmonary hypertension are consistent with our recent findings in female rats. In ovariectomized pulmonary hypertensive rats, treatment with 2ME abolished the exacerbation of disease due to removal of ovaries.[42]

As expected, in the present study monocrotaline induced significant inflammatory response. The mediators of inflammation can cause vasoconstriction and inflammatory factors/cells may contribute to vascular remodeling. Therefore, it is possible that in the present study estradiol metabolites

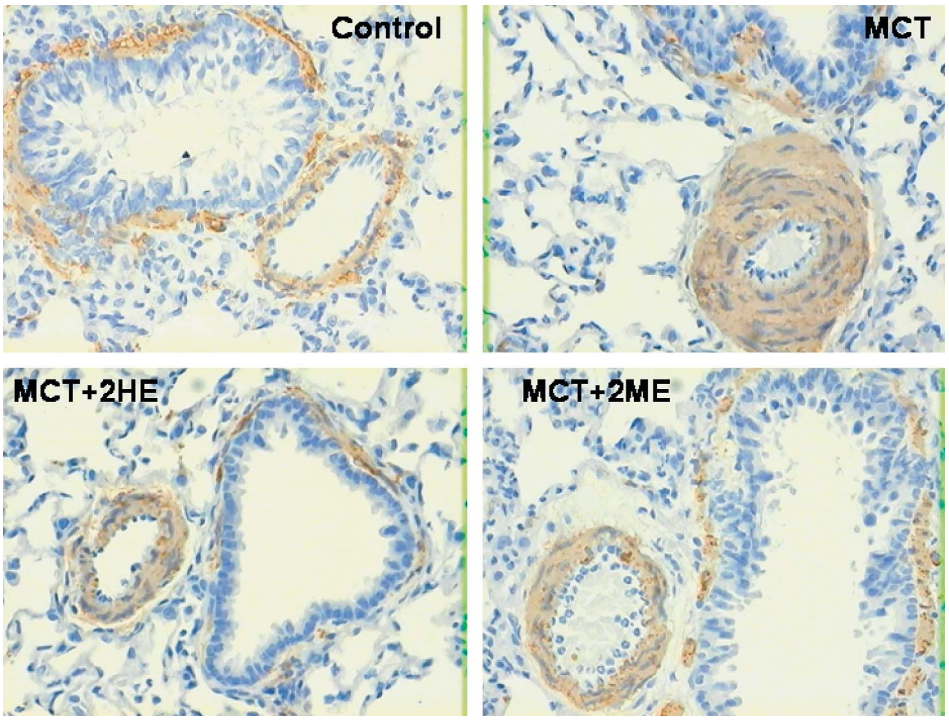

FIGURE 5. Representative immunohistochemistry (alpha actin) of small-sized pulmonary arteries in the lungs from control animal, rat with monocrotaline-induced pulmonary hypertension (MCT), and MCT rat treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME).

attenuated the development of PH by abolishing the MCT-induced inflammatory response. Nevertheless, in animals with established disease 2ME treatment (distant from the inflammatory response as an inciting event) retarded the progression of disease and markedly reduced mortality. Although inflammation was suggested as an important component in the pathobiology of pulmonary hypertension,[2] currently it is not clear whether inflammation is an inciting, resulting, and/or modulating event of vascular remodeling in human PH.[43] Monocrotaline also induced marked proliferative response in lungs, as evidenced by increased staining for PCNA. The proliferating cell nuclear antigen (PCNA) is a key protein controlling the growth state of the cell.[44] High PCNA levels in the absence of p53 indicate DNA replication, whereas high PCNA levels in the presence of p53 suggest DNA repairing. If PCNA is low or non-functional, apoptosis occurs. In the present study double staining were not performed and it is not clear which cell compartment(s) responded with proliferation and whether the reduction of proliferation by estradiol metabolites included apoptosis.

Although the objective of the present study was to evaluate the potential therapeutic effects of 2ME, the observed beneficial effects raise the question regarding the mechanism(s) of 2ME protection. The mechanisms by which 2-ME exerts its beneficial effects in PH are unknown. However, they are several mutually non-exclusive mechanisms that may account for the protective effects of 2ME in pulmonary hypertension. These include inhibition of tubulin polymerization/microtubule destabilization with arrest of mitotic cells, apoptotic effects, and anti-angiogenic effects.[22] However, at least in vitro, high concentrations of 2ME (0.5–10 μM/L) are required for producing these effects.[45–48] The strong inhibition of vascular endothelial cell proliferation may be desirable in severe PH where formation of plexiform lesions is due to tumor- like (monoclonal) endothelial cell proliferation and impaired angiogenesis.[6] Importantly, in endothelial cells, 2-ME stimulates prostacyclin synthesis, inhibits endothelin synthesis, and attenuates growth factor-induced MAPK activity.[28–30] These effects may protect the endothelium, and together with the inhibition of VSMC growth (supra vide[25–27]), may provide vascular and cardiac protection in pulmonary arterial hypertension.

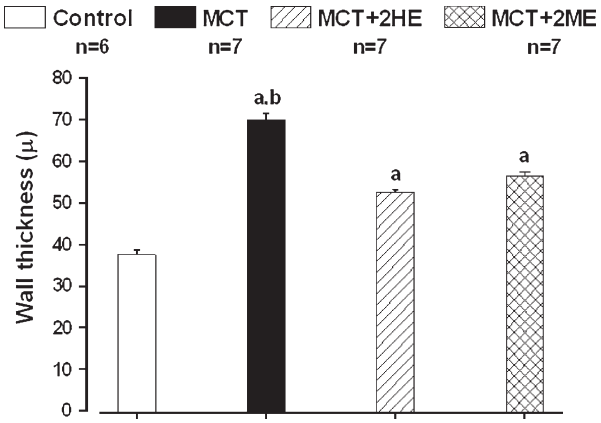

FIGURE 6. Wall thickness in small-sized pulmonary arteries (actin staining) in control animals, in rats with monocrotaline-induced pulmonary hypertension (MCT), and in MCT rats treated with 2-hydroxyestradiol (MCT+2HE) or 2-methoxyestradiol (MCT+2ME).); 1-F Anova, $P < 0.001$; Fisher LSD test for post-hoc comparison: [a]$P < 0.05$ vs. Control and MCT+2ME day 14–28 groups; [b]$P < 0.05$ vs. Control and MCT groups.

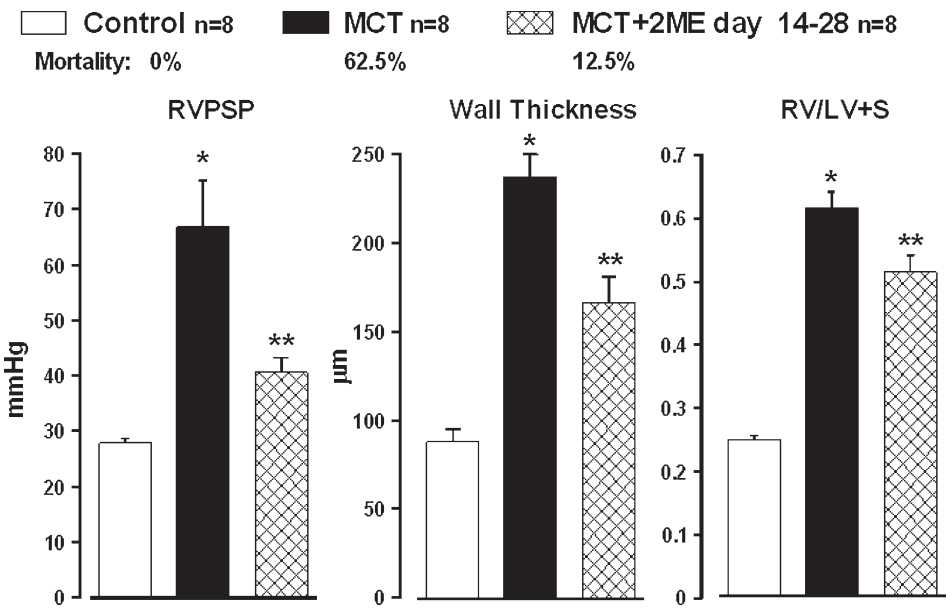

FIGURE 7. Right ventricular peak systolic pressure, RVPSP (left panel); right ventricle wall thickness of small-sized pulmonary arteries (middle panel); and left ventricle + septum ratio, RV/LV+S (right panel), in control animals (CONT), in rats with monocrotaline-induced pulmonary hypertension (MCT), and in MCT rats treated with 2-methoxyestradiol from day 14–28 after administration of MCT (MCT+2ME day 14–28 group); 1-F Anova, $P < 0.001$; Fisher LSD test for post-hoc comparison: $*P < 0.05$ vs. Control and MCT+2ME day 14–28 groups; $**P < 0.05$ vs. Control and MCT groups.

The limitations of preclinical evaluation of pharmacological agents in pulmonary hypertension apply also to this study. That is, MCT- and chronic hypoxia-induced pulmonary hypertension, the only models available, do not entirely mimic the alterations seen in humans. In both models there are no significant alterations (clustered proliferation) of the endothelium and media hypertrophy that predominates is potentially reversible upon re-exposure to normoxia or with passage of time after MCT administration. This is in contrast to the progressive character of disease in humans. Very recently, Taraseviciene-Stewart et al[49] have developed a rat model (VEGF receptor-2 antagonist plus chronic exposure to hypoxia) that mimics the key alterations seen in severe pulmonary hypertension in humans (marked pulmonary hypertension, obliterative endothelial proliferation, and media hypertrophy and progression of disease after re-exposure to normoxic environment). Therefore, further studies in this model, focused on the effects 2ME on endothelial cell function and structure (ie, endothelial remodeling), are needed to elucidate the mechanism(s) by which 2ME protects against pulmonary arterial hypertension.

In summary, this study provides the first evidence that 2ME, a major non-estrogenic, non-carcinogenic metabolite of estradiol, prevents development of monocrotaline-induced pulmonary hypertension. Furthermore, in animals with developed disease, 2ME reverses pulmonary arterial hypertension and pulmonary vascular and cardiac remodeling, and markedly reduces mortality. These beneficial effects of 2-methoxyestardiol are produced at high-physiological concentrations, (ie, concentrations that are seen in the last trimester of pregnancy) and are mediated through estrogen receptor-independent mechanism (s). Further mechanistic studies of the protective effects of 2ME, in a model of severe human-like pulmonary hypertension, are warranted.

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