Chapter

2-Methoxyestradiol in Pulmonary Arterial Hypertension: A New Disease Modifier

Stevan P. Tofovic and Edwin K. Jackson

Abstract

Pulmonary arterial hypertension (PAH), a debilitating and incurable disease, predominantly develops in women. Estradiol metabolism leads to the production of numerous metabolites with different levels of estrogenic activity and very often opposing biological effects. Dysregulated estradiol metabolism was recently linked to the penetrance, progression, and prognosis of the disease. Ongoing clinical trials are examining the effects of estradiol synthesis/signaling inhibition in patients with PAH. In this chapter, the effects of sex, sex hormones, and estradiol metabolism on the healthy pulmonary circulation and vascular pathobiology are discussed in the light of estradiol metabolism as potential pharmacological target in PAH. The effects of estrogens and their metabolites on vascular pathobiology and disease progression, their involvement in PAH-associated diseases, and the pros and cons for interventions at different levels of estradiol metabolism are discussed. Finally, we propose that 2-methoxyestradiol (2ME), a major non-estrogenic metabolite of estradiol, mediates at least in part the beneficial effects of estradiol and that 2ME exhibits opposing effects to estradiol on several processes relevant to the underlying pathophysiology of PAH, including angiogenesis, metabolic reprogramming, inflammation, and immunity. Based on cellular and in vivo effects, 2ME should be viewed as a disease modifier in women with PAH.

Keywords: pulmonary hypertension, estradiol metabolism, 2-methoxyestradiol, angiogenesis, inflammation

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive incurable disease of pulmonary vasculature that ultimately leads to failure of the right ventricle (RV) and death. Notably the disease predominantly develops in women. The first report in 1951 by Dr. David Dresdale and colleagues from Maimonides Hospital of Brooklyn on hemodynamic aspects of primary pulmonary hypertension (PH) included three 25- to 35-year-old women [1]. Similarly, in 1952 in the second seminal report on PAH, British cardiologist Paul Wood recognized that this is “...relatively rare disease, usually encountered in women between 20 and 30, but may be met at any age and in either sex...” [2]. These early observations of female preponderance of PAH were confirmed by epidemiological studies conducted in the last two decades, and the data from various registries worldwide report a female-to-male ratio (F:M) ranging
from 2:1 to 4:1 [3–9] and up to 4:1 to 9:1 for connective tissue disease [10–12]. However, with aging the female preponderance of disease disappears, and an M:F of only 1.2:1.0 has been reported in elderly PAH patients [13]. The latter strongly suggests involvement of female sex hormones in the development of PAH.

1.1 Vascular pathobiology in PAH

Pulmonary vascular remodeling is a pathological hallmark of PAH. Vascular morphological manifestations of the disease include (i) concentric and asymmetric obliterative proliferation of endothelial cells (ECs) and distal formation of multicellular plexiform lesions (PLXL); (ii) the muscularization of distal non-musculinized precapillary vessels; (iii) adventitia remodeling in form of fibrosis, inflammation, and perivascular edema; and (iv) PLXL, dilation lesions, and arteritis classified as complex lesions [14]. Three-dimensional analysis of vascular lesions in patients with severe PAH reveals the existence of two major phenotypes of ECs. The normal quiescent apoptosis-sensitive ECs are located in the peripheral areas of the lesion, are negative for phosphorylated MAPK, and have a high expression of p27kip1 (a marker of slow proliferation). The highly proliferative apoptosis-resistant cells in the central core of the vascular lesion have elevated MAPK activity and increased expression of HIF-1α, VEGF protein, and VEGF-2 receptor and low expression of p27kip1 [15, 16].

Both inflammation and immune cell response are recognized as important pathogenic factors in PAH [17–19]. For example, in experimental PH perivascular inflammation, due to macrophages, mast cells, and T and B lymphocytes, precedes vascular remodeling and elevated pulmonary pressure [20], and in PAH patients, the degree of perivascular infiltration by immune cells correlates with vascular remodeling and pulmonary artery pressure [21]. As discussed below, inflammation may markedly influence estradiol metabolism, and vice versa, estrogens and their metabolites may instigate, perpetuate, or inhibit inflammation and modulate immune cell responses in PAH.

2. Opposing effects of estradiol and 2-methoxyestradiol on estrogen metabolism

Since our first report that 2ME, a major non-estrogenic metabolite of 17β-estradiol (E2), attenuates the development and progression of monocrotaline (MCT)-induced PH and that estrogens may be pathogenic in PAH [22], a growing body of evidence suggests the involvement of dysregulated estradiol metabolism and elevated estrogen levels in the development, progression, and prognosis of PAH.

2.1 Increased estradiol production in PAH

Formation and metabolism of estrogens are complex (Figure 1). The pivotal precursors for synthesis of both androgens and estrogens are dehydroepiandrosterone (DHEA) and its biologically inactive sulfate (DHEA-S). DHEA is produced in the adrenal gland of men and postmenopausal women and in the ovaries and placenta of premenopausal women. DHEA, the most abundant steroid in circulation, is also produced by peripheral conversion from circulating DHEA-S. DHEA is transported into cells by organic anion transporters (OATPs) that are expressed in various tissues including the endothelial and inflammatory cells and lungs [23–25]. The delicate balance between DHEA and DHEA-S is controlled by the relative activity of sulfotransferase (DHEA → DHEA-S) and sulfatase (DHEA-S → DHEA) [26]. Both DHEA and DHEA-S are protective in experimental models of pulmonary
2-Methoxyestradiol in Pulmonary Arterial Hypertension: A New Disease Modifier
DOI: http://dx.doi.org/10.5772/intechopen.86812

Hypertension [27–31] including models of angioproliferative PH [32, 33]. Recently, lower DHEA-S and higher E2 levels have been linked to a greater risk of PAH and worse hemodynamics, functional status, and greater risk of death [34, 35]. Notably, DHEA improves PAH in patients with obstructive pulmonary disease [36], a finding supporting the potential therapeutic application of DHEA in PAH patients. DHEA is an over-the-counter supplement with no major side effects; however, its safety during chronic use in pharmacological doses is unknown. One potential adverse effect in this regard would be increased circulating/tissue E2 levels that may exacerbate endothelial remodeling and inflammation (infra vide).

Aromatase (encoded by the CYP19A1 gene) is the rate-limiting enzyme catalyzing the conversion of upstream androgenic precursors to estrogens (androstenedione → estrone and testosterone → E2; Figure 1). In premenopausal women, estrogens are produced predominantly in the ovarian granulosa cells and are released into the bloodstream where they act primarily in an endocrine fashion. In postmenopausal women and in men, estrogen synthesis takes place in extra-gonadal tissues (liver, heart, skin/fat tissue, and brain) where estrogens act mainly as paracrine or autocrine factors. Aromatase expression in these various sites is under the control of tissue-specific promoters regulated by different cohorts of transcription factors. Therefore, aromatase activity differs substantially in various tissues and organs in health and disease [37]. Notably, human endothelium expresses a complete aromatase-estrogen-E2 receptor system [38], and increased expression of aromatase has been reported in hPASMCs from female PAH patients and in the lungs of female rats and mice with angioproliferative PH [39]. Increased aromatase activity and plasma E2 levels seen in both men and women with advanced liver disease is associated with increased risk of portopulmonary PAH [40], and increased aromatization of androgens and elevated E2 levels have been reported in postmenopausal women and in men with PAH [34, 35]. Noteworthy, E2 stimulates aromatase activity and by increasing aromatization of androgen precursors may augment its own production as well as production of E1. Because of the importance of this enzyme in estrogen synthesis, blocking aromatase activity is an important pharmacological tool used for the treatment of estrogen-dependent diseases (breast cancer, endometriosis, and

Figure 1. Dehydroepiandrosterone is the most abundant steroid in circulation that is also produced by peripheral conversion from its circulating inactive sulfated metabolite DHEA-S. The balance between inactive sulfated sex steroids and sex steroids and their biologically active metabolites and metabolic precursors is controlled by sulfatase (STS) and sulfotransferase (SULT). Aromatase is a key enzyme in estrogen production because this enzyme controls aromatization of androgenic precursors to estrogens and intracrine production of estrogens. 2-Hydroxylation/methylation pathway of estrogen metabolism produces non-estrogenic metabolites with opposite effects to maternal estrogens. Hydroxylation of estradiol (E2) at C4 and C16 position leads to production of highly estrogenic metabolites with proliferative, pro-inflammatory, and angiogenic properties.
Anastrozole (a third-generation aromatase inhibitor) reduces E2 levels and attenuates PH in female mice exposed to hypoxia [39] and in Sugene 5416 + hypoxia rats with angioproliferative PH [39, 41]. Moreover, when combined with the selective estrogen receptor degrader fulvestrant, anastrozole reverses PH in BMPR2-mutant mice [42]. Likewise, in PAH patients treatment with anastrozole reduces elevated E2 levels by 40% and E1 levels by 70% and significantly increases functional capacity, i.e., 6-minute-walk distance [43]. Notably, E2 augments gonadal aromatase activity, and by increasing aromatization of androgens, E2 may augment its own production as well as that of other estrogens [44]. Inflammation and inflammatory cytokines upregulate aromatase activity, and TNFα is one of the most potent inducers of aromatase. In contrast to estrogens that do not have effect on TNFα induction of aromatase [45], 2ME inhibits both basal and TNFα-stimulated aromatase activity [45–47].

In addition to aromatase, another potential source of increased estrogen production in PAH is the “sulfatase pathway.” In addition to DHEA-S, other substrates for STS are biologically inactive estrone sulfate (E1-S) and estradiol sulfate (E2-S), and sulfatase plays a key role in intracrine regeneration of biologically active E2 and E1 (Figure 1). Inflammatory cytokines increase STS activity. More importantly, STS expression is stimulated by estrogens via estrogen receptor alpha (ERα) signaling, and at least in breast cancer, STS is upregulated by the elevated local E2 levels [26]. Thereby, in an inflammatory environment, E2 through feed-forward mechanisms may increase its own production via both the sulfatase and aromatase pathways (Figure 2), as implicated by elevated aromatase activity and E2 levels in both experimental PH [39] and in men and women with PAH [34, 35, 40].

2.2 Dysregulated estradiol metabolism in PAH

Once formed, E2 is primarily metabolized by oxidation at C2, C4, and C16 positions and converted to metabolites with different estrogenic activities and diverse (often opposite) biological effects. In humans, E2 hydroxylation is mediated by
multiplying CYP450 enzymes (CYP1A1/1A2/3A4/1B1) with 2-hydroxyestradiol (2HE) being the main metabolite; however, 4-hydroxyestradiol (4HE; Figures 1 and 2) is formed to a lesser degree (~5%). This is followed by methylation of hydroxyl groups catalyzed by catechol-O-methyl transferase (COMT). The hydroxylation/methylation pathway is a major metabolic pathway that accounts for ~50% of E2 metabolism. It largely takes place in the liver and leads to production of 2ME, a major non-estrogenic metabolite with antiproliferative, anti-angiogenic, and anti-inflammatory effects [48]. In addition to hepatocytes and numerous cancer cell lines, conversion of E2 to downstream 2HE and 2ME takes place in cardiovascular and renal compartments [48], and a solid line of evidence suggests that 2ME mediates the antiproliferative effects of E2 in cardiovascular and renal cells [49].

Notably, the protective effects of E2 in experimental PH are mediated, at least in part, by 2ME [50, 51]. Furthermore, it seems that in highly proliferative states, 2ME may oppose estrogen-driven proliferation. For example, in highly proliferative human leiomyoma cells (hLCs) characterized by doubled ERα signaling (inherently regulated by microtubule dynamics) [52], COMT overexpression or treatment with 2ME stabilizes microtubules, attenuates E2-induced proliferation, inhibits ERα signaling, and reduces HIF-1α and aromatase expression in hLCs [53, 54]. Unfortunately, it seems that elevated E2 levels seen in PAH may adversely affect both hepatic and extrahepatic 2ME production. In this regard, men have higher COMT activity than women [55, 56], and sex hormones regulate COMT activity that is highly expressed in human and rat lungs [57, 58]. The exposure to E2 reduces hepatic COMT activity in rats [59, 60]; in vitro E2 decreases COMT transcription, activity, and protein levels [61, 62]; and tamoxifen, by antagonizing E2, increases COMT activity in peripheral tissues [63]. Together, these findings suggest that reduced COMT activity by elevated E2 and subsequent decreased in 2ME production may render women more susceptible to the development of PAH. At present it is unknown whether there is reduced 2ME production in PAH. Yet, reduced 2ME production has been linked to the development of preeclampsia [64], increased sensitivity to angiotensin II [65], and insulin resistance [66].

2.3 Opposing effects of estradiol and 2-methoxyestradiol on CYP1B1 activity and estrogen and arachidonic acid metabolism

E2 and 2ME have opposing effects on CYP1B1, another E2 metabolizing enzyme implicated in pathogenesis of PAH. Human CYP1B1 mRNA and protein are constitutively expressed in the lung and in VSMCs and ECs [67]. CYP1B1 may facilitate E2 oxidation at C4 and C16, thus producing highly estrogenic and reactive metabolites 4-hydroxyestradiol (4HE) and 16α-hydroxyestrone (16αHE1). Experimental and human data suggest a major pathogenic role for CYP1B1 and 16αHE1 in PAH. In this regard, CYP1B1 increases the risk of PAH and RV dysfunction in humans and plays a pathogenic role in the 16αHE1-BMPR2 interaction in experimental PH [68–76]. Notably, E2 and 2ME have divergent effects on CYP1B1 activity. Estradiol is not only a substrate for CYP1B1, but also it is transcriptional activator of CYP1B1 [77]. In contrast, in vitro 2ME exerts feedback inhibition on CYP1B1 activity [78]. Moreover, 2ME inhibits aryl hydrocarbon receptor-mediated induction of CYP1B1 and reduces CYP1B1 production of reactive metabolites. In vivo, 2ME significantly inhibits CYP1B1 expression and attenuates pressure overload-induced cardiac remodeling [78, 79]. In vivo CYP1B1 inhibition by 2ME reduces biosynthesis of mid-chain hydroxyeicosatetraenoic acids (HETEs) [79], suggesting a significant role for CYP1B1 in arachidonic acid metabolism. Indeed, due to its lipoxygenase-like activity, CYP1B1 facilitates arachidonic acid metabolism into HETEs and epoxyeicosatrienoic acids (EETs) [80]. In the pulmonary vasculature, EETs and HETEs have
vasoconstrictive, inflammatory, and mitogenic/angiogenic effects and have been implicated in the development of experimental hypoxic PH [81–84]. Noteworthy, in PAH patients increased production of HETEs correlates with a poor prognosis [85]. E2 not only stimulates production of HETEs and EETs but also by inhibiting expression/activity of soluble epoxide hydrolase (sEH) [86] suppresses the degradation of EETs. Several lines of evidence link low sEH activity to the pathophysiology of PH: (1) the lungs from PH patients express no/little sEH; (2) E2-, genetic-, and pharmacologically induced downregulation of sEH potentiates hypoxic vasoconstriction; (3) hypoxia downregulates sEH; and (3) sEH−/− mice have exacerbated pulmonary vascular remodeling when exposed to chronic hypoxia [87–89]. Therefore, elevated E2 levels in PAH through a feed-forward mechanism may shift both E2 and AA metabolism toward production of pro-inflammatory/angiogenic/mitogenic metabolites. Based on its inhibitory effects, 2ME should suppress the production of these pathogenic E2 and AA metabolites (Figure 2).

3. Divergent effects of 2ME and estradiol in pulmonary endothelium in PAH

Dysregulated angiogenesis with formation of occlusive and plexiform lesions is a hallmark of PAH. Although estrogens provide protection in healthy systemic vascular beds, they have opposite effects on malignant proangiogenic/highly proliferative vessels [90] that share many similarities with vascular changes in PAH. The highly proliferative apoptosis-resistant cells in the central core of vascular lesions in PAH have elevated MAPK activity; increased expression of HIF-1α, VEGF protein, and VEGF-2 receptor; and low expression of p27kip1 (marker of low cell growth) [15, 16]. In human pulmonary artery ECs (hPAECs) and at physiological concentrations (1–10 nM), E2 (1) stimulates cell proliferation [91]; (2) promotes the phosphorylation of p42/44 and p38 MAPK via ERs; (3) downregulates the cell cycle inhibitor p27Kip1; (4) stimulates cell migration; (5) induces HIF-1α expression and VEGF synthesis; and (6) protects against apoptosis [92–94]. Also, E2 stimulates proliferation of human pulmonary artery vascular smooth muscle cells (hPASMCs). In canine pulmonary arterial segments, E2 tends to inhibit proliferation of PASMCs in segments with intact endothelium but significantly enhances proliferation in segments stripped of endothelium [95], suggesting opposite effects of E2 in intact versus injured pulmonary vessels. Thereby, in the pulmonary vasculature exposed to known and unknown multiple hits, estrogens may potentiate pathological endothelial remodeling in PAH (Figure 3).

In contrast to E2, it is well established that 2ME has strong anti-angiogenic, antiproliferative, and pro-apoptotic effects [96] and thereby may prevent PAH or inhibit the progression of PAH. In this regard, of particular importance for PAH are the effects of 2ME on the HIF-1α/VEGF axis. One of the most consistently reported effects of 2ME is HIF-1α downregulation, and 2ME has been increasingly used as pharmacological tool to inhibit HIF-1α in numerous studies outside the PAH field. HIF-1α transcriptional activity regulates more than 40 genes and respective proteins, including those that play a key role in vascular reactivity and angiogenesis [97, 98]. The role of HIF-1α in PAH is supported by multiple findings including the following: (1) oblitative endothelial lesions in severe PH in humans overexpress HIF-1α [15]; (2) in experimental PH there is similar increase in HIF-1α that correlates with the development of PH and pulmonary vascular remodeling and RV hypertrophy; (3) heterozygous deficiency in HIF-1α protects against the development of PH [99, 100]; and (4) pathologic normoxic HIF-1α signaling activation leads to the glycolytic shift (the Warburg effect) in highly proliferative ECs [101].
Hypoxia stimulates 2ME formation which inhibits the production of hypoxia-driven angiogenesis and angiogenic cytokines (VEGF and FGF-2) [31, 102]. Therefore, 2ME should be viewed as a local modulator that fine-tunes the rate of angiogenesis. Recent studies in experimental PH support the notion of 2ME as a local anti-angiogenic factor in PAH and E2 as promoter of angiogenesis. For example, (1) basal HIF-1α protein expression is higher in female hPASMCs than in males; (2) the antimitogenic effects of 2ME in hPASMCs are associated with reduced HIF-1α expression; (3) 2ME attenuates intermittent and chronic hypoxia-induced PH [22, 103, 104]; (4) in both male and female hypoxic PH rats, 2ME attenuates the disease while decreasing HIF-1α protein expression [103]; (5) female rats with Sugene 5416 + hypoxia (SU+Hx)-induced PH have more severe occlusive and plexiform lesions and sporadically develop grade 6 lesions (necrotizing arteritis); (6) E2 exacerbates angioproliferative lesions and perivascular inflammation in ovariectomized SU+Hx rats [105, 106]; and (7) in intact female SU+Hx rats, 2ME, but not E2, exhibits therapeutic effects [107]. The effects of 2ME in PAH patients are unknown. Yet, at least in experimental angioproliferative PH, 2ME could be viewed as biological antagonist of E2 in the endothelium and as a modifier of “dysregulated angiogenesis.”

4. Metabolic reprogramming and 2ME in PAH

The major metabolic changes that take place in PAH occur in the form of the shift from oxidative phosphorylation to glycolysis. Known as the Warburg effect, this event is frequently observed and has been systematically investigated in cancer tissue. Notably, the Warburg effect has also been reported in pulmonary vasculature cells in PAH patients [108] and linked to highly proliferative, angiogenic, and apoptosis-resistant cancer cells and vascular cells in PAH. Not surprisingly, the
Warburg effect has been explored as a potential anti-angiogenic target in cancer and more recently in PAH. The HIF-1α transcription factor has been identified as a master hypoxic regulator responsible for the metabolic shift in PAH [101, 109]. Hypoxic induction of HIF-1α leads to overexpression of pyruvate dehydrogenase kinase (PDK) which results in inhibition of pyruvate dehydrogenase that shunts pyruvate into glycolysis and induces conversion of glucose to lactate [108]. Dichloroacetate (DCA), a PDK inhibitor, reverses the Warburg effect and exhibits therapeutic effects in several animal models of PH [110–112]. Because 2ME is a strong HIF-1α inhibitor, 2ME should induce metabolic reprogramming in PAH. Presently, the effects of 2ME on metabolic reprogramming in PAH are unknown. However, 2ME inhibits lactate-induced mitochondrial biogenesis in highly proliferative osteosarcoma cells, and in apoptosis-resistant melanoma cells, 2ME attenuates proliferation and glycolysis by inhibiting HIF-1α and PDK expression [113–115]. Therefore, 2ME could be viewed as modulator of metabolic reprogramming. Further studies are warranted to investigate the effects of 2ME on the Warburg effect that in PAH is associated with highly proliferative, angiogenic, and apoptosis-resistant phenotypes (Figure 4).

5. Anti-inflammatory and immunomodulatory effects of 2ME

Inflammation and altered immunity, i.e., perivascular accumulation of inflammatory and immune cells in pulmonary circulation, have been increasingly recognized as pathogenic factors in PAH [17–19]. In this regard, at young age women have more robust immune responses than men. Although initially beneficial, with aging these aggressive immune responses may become detrimental [116]. This may explain why various immune diseases are remarkably more frequent in women and why many immune diseases, such as systemic sclerosis (SSc), lupus, and mixed connective tissue disease, are associated with increased risk of PAH [117]. Furthermore, recently distinct immune phenotypes have been reported in PAH patients [118]. In experimental PH, dysregulated immunity in the form of deficient regulatory T-cell (Treg) activity contributes to increased inflammation [20]. Both alveolar macrophages and immune cells express steroidogenic enzymes including sulfatase and aromatase [119–121]. Inflammatory cytokines, prostanooids, and growth factors regulate the expression and activity of steroidogenic enzymes, and in turn, sex hormones
may influence the production and release of these autocrine/paracrine mediators \[122\]. E2 upregulates CYP1B1, aromatase, and sulfatase activity and inhibits sEH activity. Therefore, in an inflammatory environment, E2 may boost its own production, and via a feed-forward mechanism, E2 may enhance the production of pro-inflammatory, angiogenic, and mitogenic estrogens and increase the accumulation of pro-inflammatory arachidonic acid metabolites (Figure 2). In contrast to E2, non-estrogenic 2ME exhibits significant anti-inflammatory effects, largely through suppression of tissue recruitment and activation of macrophages \[123, 124\]. This is one of the most consistent in vivo effects of 2ME seen in experimental models of cardiovascular and renal injury \[125, 126\] and in pulmonary hypertension \[50, 51, 127–129\]. 2ME, its metabolic precursor 2HE, and the synthetic analog 2-ethoxyestradiol inhibit influx and activation of macrophages in MCT- and bleomycin-induced PH, and this inhibition correlates with reduced PH, vascular remodeling, and fibrosis. 2ME and its metabolic precursor 2HE also inhibit the synthesis of leukotrienes \[130\]. Blocking of leukotriene production by macrophages prevents endothelial injury and reverses experimental PH \[131\]. In experimental autoimmune rheumatoid arthritis, 2ME slows down disease progression by inhibiting inflammatory cytokine mRNA (IL-1β, TNF-α, IL-6, and IL-17), leucocyte infiltration, and neovascularization \[31\]. In several models of autoimmune inflammatory disease, the beneficial effects of 2ME were ascribed to the inhibition of immune cell activation, proliferation, and pro-inflammatory cytokine release \[31, 132–134\]. Finally, in fibroblasts from SSc disease patients that are at high risk for developing PAH, 2ME reduces hypoxia-induced production of connective tissue growth factor and collagen I by inhibiting the PI3K/Akt/mTOR or HIFα signaling \[135\]. Collectively, these data in inflammatory and autoimmune diseases point toward 2ME as potential modulator of inflammation and immunity relevant to the development and progression of PAH.

6. 2-Methoxyestradiol and the renin-angiotensin system (RAS) in PAH

Evidence suggests that the renin-angiotensin system (RAS) contributes to the development of PAH \[136, 137\]. For example, (1) there is increased systemic RAS activity in patients with idiopathic PAH; (2) in experimental and human PH, ACE activity and expression are increased in PAECs, PVSMCs, plexiform lesions, and the RV; (3) increased Ang II type 1 receptor expression and signaling correlates with PAH progression and vascular remodeling \[137–142\]; and (4) inhibition of RAS slows down the progression of MCT-induced PH \[143\]. Cumulating data also suggests that 2ME may behave as biological antagonist of Ang II. 2ME downregulates Ang II type I receptors \[144–146\]. In COMT−/− mice that have reduced 2ME production, 2ME treatment abolishes hypersensitivity to and injury induced by Ang II \[65\]. Furthermore, in Cyp1B1−/− mice that also have reduced 2ME production, 2ME treatment abolishes Ang II-induced oxidative and vascular injury \[147\]. Finally, of relevance in PAH, in vivo in high RAS activity models, 2ME attenuates Ang II-induced cardiac and vascular remodeling and fibrosis and isoproterenol-induced RV and LV hypertrophy and fibrosis \[148\].

7. Role of 2ME and the metabolic syndrome in PAH

The metabolic syndrome (MS) is recognized as risk factor for PH \[149, 150\]. Deficiency in PPARγ (a downstream target of BMPR2) and deficiency in apolipoprotein E and adiponectin (downstream targets of PPARγ) have been linked to the development of PH in rodents \[151–153\]. Moreover, COMT, via methoxyestradiols,
has been identified as a major factor modulating insulin resistance. The low-activity COMT Val-Met is linked to MS [154], whereas high-activity COMT rs4680 is associated with lower HbA1c levels and protection from type 2 diabetes [155]. COMT deficiency in mice leads to disrupted glucose homeostasis [66], and 2ME which shares structural similarity with PPARγ ligands and acts as a PPARγ agonist [65, 156] (Figure 4) induces AMPK phosphorylation and improves insulin sensitivity in COMT−/− mice [66]. 2HE, a metabolic precursor of 2ME and COMT substrate, activates AMPK in human skeletal muscle, attenuates experimental PH in lean rats, and reduces MS-induced endothelial dysfunction in obese rats. Moreover, in rats with polygenic obesity and MS, in both PH-free females and PH male ZDSD rats, treatment with 2HE reduces glycosylated hemoglobin, RVSP, and RV-EDP and attenuates vascular remodeling in male PH rats [157]. These data warrant further investigation of 2ME in MS-induced PH and support the notion of 2ME as potential disease modifier in MS-related PH.

8. 2-Methoxyestradiol and current pharmacotherapy of PAH

Despite significant advances in pharmacotherapy of PAH, mortality of patients with PAH remains high. Therefore, there is still a significant unmet medical need for more effective therapies. Currently approved drugs for treatment of PAH include medications that correct for prostanoid deficiency (prostanoids and prostacyclin receptor agonists) and deficiency of nitric oxide (PDE5 inhibitors and soluble guanylate cyclase stimulators) or combat overproduction of endothelin (endothelin receptor antagonists). Compared to ECs in healthy vessels, the ECs in affected vessels in PH show reduced prostacyclin and nitric oxide synthesis and overexpression of ET-1 [158–160]. Noteworthy, compared to estradiol, 2ME is a more potent inhibitor of endothelin synthesis in endothelial cells [48, 161], and 2ME and its metabolic precursor 2HE inhibit endothelin-induced vasoconstriction [162]. Furthermore, in ECs 2ME is a more potent stimulator of prostacyclin synthesis than estradiol [163, 164]. 2ME also increases basal and potentiates stimulated NO production in male and OVX female rats, but has no effect in intact females, and in vitro these effects are abolished by the eNOS inhibitor L-NAME [165]. 2ME induces vasodilation by stimulating NO release via PPARγ/P3K/Akt pathway [166] and increases NO production in uterine artery ECs from pregnant sheep [167]. Moreover, in L-NAME-treated rats, 2ME attenuates severe hypertension and renal, cardiac, and vascular injury and inflammation and reduces mortality by 87% [125]. Likewise, 2ME exhibits beneficial effects in MCT-induced PH and efficacy comparable to that of bosentan and sildenafil. Importantly, in combination with bosentan or sildenafil, 2ME has synergistic therapeutic effects (further reduces vascular remodeling, inflammatory responses, and survival) [128]. Finally, none of the approved therapies for PAH affects endothelial remodeling and “dysregulated angiogenesis” in pulmonary vasculature; in contrast, as discussed above in Section 3, 2ME is a strong anti-angiogenic agent and inhibitor of HIF-VEGF axis that is critical for the metabolic shift and development of occlusive and complex vascular lesions. Altogether, these data clearly indicate 2ME as a promising pharmacological agent capable of providing additional benefit in PAH patients on standard single or combination therapy.

9. Pharmacokinetic aspects of the development of 2ME as a disease modifier in PAH

The safety and efficacy of 2ME in human PAH are unknown. However, over the past two decades, numerous phase I and II clinical trials have been conducted to test
the safety and antitumor efficacy of 2ME in patients with solid malignancies. These studies show that 2ME is well tolerated and safe in doses up to 3 g/day. Unfortunately, even high oral doses of 2ME achieve only low plasma 2ME concentrations due to high pre-systemic metabolism (glucuronidation) in the liver. In experimental PH, therapeutic effects of 2ME are achieved by much lower doses (240 μg/kg/day) delivered by subcutaneous micro-infusions that produce high physiological concentrations of 2ME (~3 ng/ml; equivalent to levels observed during the last trimester of pregnancy) [168]. At these concentrations, 2ME does not induce estrogenic effects. In rats with MCT-induced PH, although higher doses of 2ME do not additionally reduce PH and RV hypertrophy, higher doses do further inhibit media remodeling and inflammation [127]. Likewise, in contrast to oral administration in healthy volunteers and cancer patients, subcutaneous administration of a long-acting formulation of 2ME in doses up to 10 mg produced blood levels of 2ME >1 ng/ml over a 3-week period, with no estrogenic or other adverse effects reported [169]. Currently, various parenteral formulations of 2ME with supposedly high bioavailability are under investigation.

10. Conclusions and future directions

An expanding body of knowledge indicates that many of the beneficial cellular and systemic effects of E2 are due, at least in part, to its major and non-estrogenic metabolite 2ME. This underscores the importance of estradiol metabolism to 2ME in women’s health and suggests that 2ME deficiency may contribute to many female predominant diseases, including PAH. 2ME should not be viewed only as partial mediator of E2 effects but in PAH should be considered a moderator of the harmful effects of estrogens related to several key events in PAH including altered estradiol and arachidonic acid metabolism, angiogenesis, inflammation, harmful immune responses, metabolic syndrome, and metabolic reprogramming. The above discussion hopefully makes the case for 2ME as unrecognized disease modifier in PAH.

Conflict of interest

The authors do not have any conflict of interest.
Author details

Stevan P. Tofovic\(^{1,2,3}\) and Edwin K. Jackson\(^2\)

1 Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

2 Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

3 Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

*Address all correspondence to: tofovic@pitt.edu

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
2-Methoxyestradiol in Pulmonary Arterial Hypertension: A New Disease Modifier
DOI: http://dx.doi.org/10.5772/intechopen.86812

References

[1] Dresdale DT, Schultz M, Michtom RJ. Primary pulmonary hypertension. I. Clinical and hemodynamic study. American Journal of Medicine. 1951;11:686-705

[2] Wood P. Pulmonary hypertension. British Medical Bulletin. 1952;8:348-353

[3] Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL registry. Chest. 2012;142:448-456. DOI: 10.1378/chest.11-1460

[4] Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, et al. Predicting survival in pulmonary arterial hypertension: Insights from the registry to evaluate early and long-term pulmonary arterial hypertension disease management (REVEAL). Circulation. 2010;122:164-172. DOI: 10.1161/CIRCULATIONAHA.109.898122

[5] Escribano-Subias P, Blanco I, Lopez-Meseguer M, Lopez-Guarch CJ, Roman A, Morales P, et al. Survival in pulmonary hypertension in Spain: Insights from the spanish registry. The European Respiratory Journal. 2012;40:596-603. DOI: 10.1183/09031936.00101211

[6] Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation. 2010;122:156-163. DOI: 10.1161/CIRCULATIONAHA.109.911818

[7] Humbert M, Sitbon O, Yaici A, Montani D, O’Callaghan DS, Jais X, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. The European Respiratory Journal. 2010;36:549-555. DOI: 10.1183/09031936.00057010

[8] Jing ZC, Xu XQ, Han ZY, Wu Y, Deng KW, Wang H, et al. Registry and survival study in Chinese patients with idiopathic and familial pulmonary arterial hypertension. Chest. 2007;132:373-379. DOI: 10.1378/chest.06-2913

[9] Tamura Y, Kumamaru H, Satoh T, Miyata H, Ogawa A, Tanabe N, et al. Effectiveness and outcome of pulmonary arterial hypertension-specific therapy in Japanese patients with pulmonary arterial hypertension. Circulation Journal: Official Journal of the Japanese Circulation Society. 2017;82:275-282. DOI: 10.1253/circj.CJ-17-0139

[10] Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: Results from a national registry. American Journal of Respiratory and Critical Care Medicine. 2006;173:1023-1030. DOI: 10.1164/rcrm.200510-1668OC

[11] McGoon MD, Miller DP. REVEAL: A contemporary US pulmonary arterial hypertension registry. European Respiratory Review: An Official Journal of the European Respiratory Society. 2012;21:8-18. DOI: 10.1183/09059180.0008211

[12] Zhang R, Dai LZ, Xie WP, Yu ZX, Wu BX, Pan L, et al. Survival of Chinese patients with pulmonary arterial hypertension in the modern treatment era. Chest. 2011;140:301-309. DOI: 10.1378/chest.10-2327

[13] Hoeper MM, Huscher D, Ghofrani HA, Delcroix M, Distler O, Schweiger C, et al. Elderly patients diagnosed with idiopathic pulmonary arterial hypertension: Results from the
COMPERA registry. International Journal of Cardiology. 2013;168:871-880. DOI: 10.1016/j.ijcard.2012.10.026

[14] Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. Journal of the American College of Cardiology. 2004;43:25S-32S. DOI: 10.1016/j.jacc.2004.02.033

[15] Cool CD, Stewart JS, Werahera P, Miller GJ, Williams RL, Voelkel NF, et al. Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. Evidence for a dynamic and heterogeneous process of pulmonary endothelial cell growth. The American Journal of Pathology. 1999;155:411-419. DOI: 10.1016/S0002-9440(10)65137-1

[16] Tuder RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: Evidence for a process of disordered angiogenesis. The Journal of Pathology. 2001;195:367-374. DOI: 10.1002/path.953

[17] Hassoun PM, Mouthon L, Barbera JA, Eddahibi S, Flores SC, Grimminger F, et al. Inflammation, growth factors, and pulmonary vascular remodeling. Journal of the American College of Cardiology. 2009;54:S10-S19. DOI: 10.1016/j.jacc.2009.04.006

[18] Kuebler WM, Bonnet S, Tabuchi A. Inflammation and autoimmunity in pulmonary hypertension: Is there a role for endothelial adhesion molecules? (2017 Grover conference series). Pulmonary Circulation. 2018;8:2045893218757596. DOI: 10.1177/2045893218757596

[19] Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. Circulation Research. 2014;115:165-175. DOI: 10.1161/CIRCRESAHA.113.301141

[20] Tamosiuniene R, Tian W, Dhillon G, Wang L, Sung YK, Gera L, et al. Regulatory T cells limit vascular endothelial injury and prevent pulmonary hypertension. Circulation Research. 2011;109:867-879. DOI: 10.1161/CIRCRESAHA.110.236927

[21] Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, et al. Modern age pathology of pulmonary arterial hypertension. American Journal of Respiratory and Critical Care Medicine. 2012;186:261-272. DOI: 10.1164/rccm.201201-0164OC

[22] Tofovic SP, Zhang X, Jones T, Jackson EK, Petresvsk G. 2-Methoxyestradiol attenuates the development and retards the progression of chronic hypoxia-induced pulmonary hypertension in rats. Circulation. 2005;112:98-99

[23] Mikkaichi T, Suzuki T, Tanemoto M, Ito S, Abe T. The organic anion transporter (OATP) family. Drug Metabolism and Pharmacokinetics. 2004;19:171-179

[24] Obaidat A, Roth M, Hagenbuch B. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. Annual Review of Pharmacology and Toxicology. 2012;52:135-151. DOI: 10.1146/annurev-pharmtox-010510-100556

[25] Radford DJ, Wang K, McNelis JC, Taylor AE, Hechenberger G, Hofmann J, et al. Dehydroepiandrosterone sulfate directly activates protein kinase C-beta to increase human neutrophil superoxide generation. Molecular Endocrinology. 2010;24:813-821. DOI: 10.1210/me.2009-0390
ventricular structure and function in rats with severe pulmonary arterial hypertension. American Journal of Physiology Heart and Circulatory Physiology. 2013;304:H1708-H1718. DOI: 10.1152/ajpheart.00746.2012

[33] Homma N, Nagaoka T, Karoor V, Imamura M, Taraseviciene-Stewart L, Walker LA, et al. Involvement of RhoA/Rho kinase signaling in protection against monocrotaline-induced pulmonary hypertension in pneumonecтомized rats by dehydroepiandrosterone. American Journal of Physiology Lung Cellular and Molecular Physiology. 2008;295:L71-L78. DOI: 10.1152/ajplung.90251.2008

[34] Baird GL, Archer-Chicko C, Barr RG, Bluemke DA, Foderaro AE, Fritz JS, et al. Lower DHEA-S levels predict disease and worse outcomes in post-menopausal women with idiopathic, connective tissue disease- and congenital heart disease-associated pulmonary arterial hypertension. The European Respiratory Journal. 2018;51(6). DOI: 10.1183/13993003.00467-2018

[35] Ventetuolo CE, Baird GL, Barr RG, Bluemke DA, Fritz JS, Hill NS, et al. Higher estradiol and lower dehydroepiandrosterone-sulfate levels are associated with pulmonary arterial hypertension in men. American Journal of Respiratory and Critical Care Medicine. 2016;193:1168-1175. DOI: 10.1164/rccm.201509-1785OC

[36] Dumas de La Roque E, Savineau JP, Metivier AC, Billes MA, Kraemer JP, Doutreleau S, et al. Dehydroepiandrosterone (DHEA) improves pulmonary hypertension in chronic obstructive pulmonary disease (COPD): A pilot study. Annales D’endocrinologie. 2012;73:20-25. DOI: 10.1016/j.ando.2011.12.005

[37] Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood
MM, et al. Aromatase expression in health and disease. Recent Progress in Hormone Research. 1997;52:185-213 discussion 213-4

[38] Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, et al. Aromatase—A brief overview. Annual Review of Physiology. 2002;64:93-127. DOI: 10.1146/annurev.physiol.64.081601.142703

[39] Mair KM, Wright AF, Duggan N, Rowlands DJ, Hussey MJ, Roberts S, et al. Sex-dependent influence of endogenous estrogen in pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine. 2014;190:456-467. DOI: 10.1164/rccm.201403-0483OC

[40] Roberts KE, Fallon MB, Krowka MJ, Brown RS, Trotter JF, Peter I, et al. Genetic risk factors for portopulmonary hypertension in patients with advanced liver disease. American Journal of Respiratory and Critical Care Medicine. 2009;179:835-842. DOI: 10.1164/rccm.200809-1472OC

[41] Tofovic S, Bilan V, Mi Z, Jackson EK, Schneider F. Aromatase inhibition attenuates and ovariectomy and 4-hydroxyestradiol have mixed effects on development of angioproliferative pulmonary hypertension in female rats. American Journal of Respiratory and Critical Care Medicine. 2013;187:A6099

[42] Chen X, Austin ED, Talati M, Fessel JP, Farber-Eger EH, Brittain EL, et al. Oestrogen inhibition reverses pulmonary arterial hypertension and associated metabolic defects. The European Respiratory Journal. 2017;50. DOI: 10.1183/13993003.02337-2016

[43] Kawut SM, Archer-Chicko CL, DeMichele A, Fritz JS, Klinger JR, Ky B, et al. Anastrozole in pulmonary arterial hypertension. A randomized, double-blind, placebo-controlled trial. American Journal of Respiratory and Critical Care Medicine. 2017;195:360-368. DOI: 10.1164/rccm.201605-1024OC

[44] Adashi EY, Hsueh AJ. Estrogens augment the stimulation of ovarian aromatase activity by follicle-stimulating hormone in cultured rat granulosa cells. The Journal of Biological Chemistry. 1982;257:6077-6083

[45] Schmidt M, Hartung R, Capellino S, Cutolo M, Pfeifer-Leeg A, Straub RH. Estrone/17beta-estradiol conversion to, and tumor necrosis factor inhibition by, estrogen metabolites in synovial cells of patients with rheumatoid arthritis and patients with osteoarthritis. Arthritis and Rheumatism. 2009;60:2913-2922. DOI: 10.1002/art.24859

[46] Capellino S, Straub RH, Cutolo M. Aromatase and regulation of the estrogen-to-androgen ratio in synovial tissue inflammation: Common pathway in both sexes. Annals of the New York Academy of Sciences. 2014;1317:24-31. DOI: 10.1111/nyas.12398

[47] Purohit A, Singh A, Ghilchik MW, Reed MJ. Inhibition of tumor necrosis factor alpha-stimulated aromatase activity by microtubule-stabilizing agents, paclitaxel and 2-methoxyestradiol. Biochemical and Biophysical Research Communications. 1999;261:214-217. DOI: 10.1006/bbrc.1999.1010

[48] Dubey RK, Tofovic SP, Jackson EK. Cardiovascular pharmacology of estradiol metabolites. The Journal of Pharmacology and Experimental Therapeutics. 2004;308:403-409. DOI: 10.1124/jpet.103.058057

[49] Dubey RK, Imthurn B, Jackson EK. 2-methoxyestradiol: A potential treatment for multiple proliferative disorders. Endocrinology. 2007;148:4125-4127. DOI: 10.1210/en.2007-0514

[50] Tofovic SP, Zhang X, Jackson EK, Dacic S, Petruesevska G.
2-methoxyestradiol mediates the protective effects of estradiol in monocrotaline-induced pulmonary hypertension. Vascular Pharmacology. 2006;45:358-367. DOI: 10.1016/j.vph.2006.05.007

[51] Tofovic SP, Zhang X, Jackson EK, Zhu H, Petreuevska G. 2-methoxyestradiol attenuates bleomycin-induced pulmonary hypertension and fibrosis in estrogen-deficient rats. Vascular Pharmacology. 2009;51:190-197. DOI: 10.1016/j.vph.2009.06.002

[52] Manavathi B, Acconcia F, Rayala SK, Kumar R. An inherent role of microtubule network in the action of nuclear receptor. Proceedings of the National Academy of Sciences of the United States of America. 2006;103:15981-15986. DOI: 10.1073/pnas.0607445103

[53] Salama SA, Kamel MW, Botting S, Salih SM, Borahay MA, Hamed AA, et al. Catechol-o-methyltransferase expression and 2-methoxyestradiol affect microtubule dynamics and modify steroid receptor signaling in leiomyoma cells. PLoS One. 2009;4:e7356. DOI: 10.1371/journal.pone.0007356

[54] Salama SA, Nasr AB, Dubey RK, Al-Hendy A. Estrogen metabolite 2-methoxyestradiol induces apoptosis and inhibits cell proliferation and collagen production in rat and human leiomyoma cells: A potential medicinal treatment for uterine fibroids. Journal of the Society for Gynecologic Investigation. 2006;13:542-550. DOI: 10.1016/j.jsgi.2006.09.003

[55] Boudikova B, Szumlanski C, Maidak B, Weinshilboim R. Human liver catechol-O-methyltransferase pharmacogenetics. Clinical Pharmacology and Therapeutics. 1990;48:381-389

[56] Floderus Y, Ross SB, Wetterberg L. Erythrocyte catechol-O-methyltransferase activity in a Swedish population. Clinical Genetics. 1981;19:389-392

[57] Bryan-Lluka LJ. Evidence for saturation of catechol-O-methyltransferase by low concentrations of noradrenaline in perfused lungs of rats. Naunyn-Schmiedeberg’s Archives of Pharmacology. 1995;351:408-416

[58] De Santi C, Giulianotti PC, Pietrabissa A, Mosca F, Pacifici GM. Catechol-O-methyltransferase: Variation in enzyme activity and inhibition by entacapone and tolcapone. European Journal of Clinical Pharmacology. 1998;54:215-219

[59] Cohn CK, Axelrod J. The effect of estradiol on catechol-O-methyltransferase activity in rat liver. Life Sciences Pt. 1: Physiology and Pharmacology. 1971;10:1351-1354

[60] Parvez S, Parvez SH, Youdim MB. Variation in activity of monoamine metabolizing enzymes in rat liver during pregnancy. British Journal of Pharmacology. 1975;53:241-246

[61] Jiang H, Xie T, Ramsden DB, Ho SL. Human catechol-O-methyltransferase down-regulation by estradiol. Neuropharmacology. 2003;45:1011-1018

[62] Xie T, Ho SL, Ramsden D. Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. Molecular Pharmacology. 1999;56:31-38

[63] Schendzielorz N, Rysa A, Reenila I, Raasmaja A, Mannisto PT. Complex estrogenic regulation of catechol-O-methyltransferase (COMT) in rats. Journal of Physiology and Pharmacology: An Official Journal
of the Polish Physiological Society. 2011;62:483-490

[64] Wantania J, Attamimi A, Siswishanto R. A comparison of 2-methoxyestradiol value in women with severe preeclampsia versus normotensive pregnancy. Journal of Clinical and Diagnostic Research: JCDR. 2017;11:QC35-QC38. DOI: 10.7860/JCDR/2017/21516.9603

[65] Ueki N, Kanasaki K, Kanasaki M, Takeda S, Koya D. Catechol-O-methyltransferase deficiency leads to hypersensitivity of the pressor response against angiotensin II. Hypertension. 2017;69:1156-1164. DOI: 10.1161/HYPERTENSIONAHA.117.09247

[66] Kanasaki M, Srivastava SP, Yang F, Xu L, Kudoh S, Kitada M, et al. Deficiency in catechol-o-methyltransferase is linked to a disruption of glucose homeostasis in mice. Scientific Reports. 2017;7:7927. DOI: 10.1038/s41598-017-08513-w

[67] Kerzee JK, Ramos KS. Constitutive and inducible expression of Cyp1a1 and Cyp1b1 in vascular smooth muscle cells: Role of the Ahr bHLH/PAS transcription factor. Circulation Research. 2001;89:573-582

[68] Austin ED, Cogan JD, West JD, Hedges LK, Hamid R, Dawson EP, et al. Alterations in oestrogen metabolism: Implications for higher penetrance of familial pulmonary arterial hypertension in females. The European Respiratory Journal. 2009;34:1093-1099. DOI: 10.1183/09031936.00010409

[69] Chen X, Talati M, Fessel JP, Hemnes AR, Gladson S, French J, et al. Estrogen metabolite 16alpha-hydroxyestrone exacerbates bone morphogenetic protein receptor type II-associated pulmonary arterial hypertension through MicroRNA-29-mediated modulation of cellular metabolism. Circulation. 2016;133:82-97. DOI: 10.1161/CIRCULATIONAHA.115.016133

[70] Hood KY, Montezano AC, Harvey AP, Nilsen M, MacLean MR, Touyz RM. Nicotinamide adenine dinucleotide phosphate oxidase-mediated redox signaling and vascular remodeling by 16alpha-hydroxyestrone in human pulmonary artery cells: Implications in pulmonary arterial hypertension. Hypertension. 2016;68:796-808. DOI: 10.1161/HYPERTENSIONAHA.116.07668

[71] Johansen AK, Dean A, Morecroft I, Hood K, Nilsen M, Loughlin L, et al. The serotonin transporter promotes a pathological estrogen metabolic pathway in pulmonary hypertension via cytochrome P450 1B1. Pulmonary Circulation. 2016;6:82-92. DOI: 10.1086/685023

[72] Loyd JE. Pulmonary arterial hypertension: Insights from genetic studies. Proceedings of the American Thoracic Society. 2011;8:154-157. DOI: 10.1513/pats.201007-047MS

[73] Ventetuolo CE, Mitra N, Wan F, Manichaikul A, Barr RG, Johnson C, et al. Oestradiol metabolism and androgen receptor genotypes are associated with right ventricular function. The European Respiratory Journal. 2016;47:553-563. DOI: 10.1183/13993003.01083-2015

[74] Ventetuolo CE, Ouyang P, Bluemke DA, Tandri H, Barr RG, Bagiella E, et al. Sex hormones are associated with right ventricular structure and function: The MESA-right ventricle study. American Journal of Respiratory and Critical Care Medicine. 2011;183:659-667. DOI: 10.1164/rccm.201007-1027OC

[75] West J, Cogan J, Geraci M, Robinson L, Newman J, Phillips JA, et al. Gene expression in BMPR2 mutation carriers with and without evidence of pulmonary arterial hypertension suggests pathways relevant to disease penetrance. BMC Medical Genomics. 2008;1:45. DOI: 10.1186/1755-8794-1-45
[76] White K, Johansen AK, Nilsen M, Ciuclan L, Wallace E, Paton L, et al. Activity of the estrogen-metabolizing enzyme cytochrome P450 1B1 influences the development of pulmonary arterial hypertension. Circulation. 2012;126:1087-1098. DOI: 10.1161/CIRCULATIONAHA.111.062927

[77] Tsuchiya Y, Nakajima M, Yoko T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Letters. 2005;227:115-124. DOI: 10.1016/j.canlet.2004.10.007

[78] Dawling S, Roodi N, Parl FF. Methoxyestrogens exert feedback inhibition on cytochrome P450 1A1 and 1B1. Cancer Research. 2003;63:3127-3132

[79] Maayah ZH, Levasseur J, Siva Piragasam R, Abdelhamid G, Dyck JRB, Fahlman RP, et al. 2-methoxyestradiol protects against pressure overload-induced left ventricular hypertrophy. Scientific Reports. 2018;8:2780. DOI: 10.1038/s41598-018-20613-9

[80] Choudhary D, Jansson I, Stoilov I, Sarfarazi M, Schenkman JB. Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1B1. Drug Metabolism and Disposition: The Biological Fate of Chemicals. 2004;32:840-847

[81] Jiang J, Wang S, Wang Z, Ma J, Liu S, Li W, et al. The role of ERK1/2 in 15-HETE-inhibited apoptosis in pulmonary arterial smooth muscle cells. Journal of Receptor and Signal Transduction Research. 2011;31:45-52. DOI: 10.3109/10799893.2010.512013

[82] Ma C, Li Y, Ma J, Liu Y, Li Q, Niu S, et al. Key role of 15-lipoxygenase/15-hydroxyeicosatetraenoic acid in pulmonary vascular remodeling and vascular angiogenesis associated with hypoxic pulmonary hypertension. Hypertension. 2011;58:679-688. DOI: 10.1161/HYPERTENSIONAHA.111.171561

[83] Maayah ZH, Althurwi HN, Abdelhamid G, Lesyk G, Jurasz P, El-Kadi AO. CYP1B1 inhibition attenuates doxorubicin-induced cardiotoxicity through a mid-chain HETEs-dependent mechanism. Pharmacological Research. 2016;105:28-43. DOI: 10.1016/j.phrs.2015.12.016

[84] Sugumaran PK, Wang S, Song S, Nie X, Zhang L, Feng Y, et al. 15-oxo-eicosatetraenoic acid prevents serum deprivation-induced apoptosis of pulmonary arterial smooth muscle cells by activating pro-survival pathway. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2014;90:89-98. DOI: 10.1016/j.pla.2014.01.006

[85] Al-Naamani N, Sagliani KD, Dolnikowski GG, Warburton RR, Toksoz D, Kayyali U, et al. Plasma 12- and 15-hydroxyeicosanoids are predictors of survival in pulmonary arterial hypertension. Pulmonary Circulation. 2016;6:224-233. DOI: 10.1086/686311

[86] Yang YM, Sun D, Kandhi S, Froogh G, Zhuge J, Huang W, et al. Estrogen-dependent epigenetic regulation of soluble epoxide hydrolase via DNA methylation. Proceedings of the National Academy of Sciences of the United States of America. 2018;115:613-618. DOI: 10.1073/pnas.1716016115

[87] Kandhi S, Qin J, Froogh G, Jiang H, Luo M, Wolin MS, et al. EET-dependent potentiation of pulmonary arterial pressure: Sex-different regulation of soluble epoxide hydrolase. American Journal of Physiology Lung Cellular and Molecular Physiology. 2015;309:L1478-L1486. DOI: 10.1152/ajplung.00208.2015

[88] Kandhi S, Zhang B, Froogh G, Qin J, Alruwaili N, Le Y, et al. EETs promote hypoxic pulmonary vasoconstriction via constrictor prostanoids. American Journal of Physiology Lung Cellular and Molecular Physiology.
[89] Keseru B, Barbosa-Sicard E, Schermuly RT, Tanaka H, Hammock BD, Weissmann N, et al. Hypoxia-induced pulmonary hypertension: Comparison of soluble epoxide hydrolase deletion vs. inhibition. Cardiovascular Research. 2010;85: 232-240. DOI: 10.1093/cvr/cvp281

[90] Klouche M. Estrogens in human vascular diseases. Annals of the New York Academy of Sciences. 2006;1089:431-443. DOI: 10.1196/annals.1386.032

[91] Lippert C, Seeger H, Mueck AO, Lippert TH. The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells. Life Sciences. 2000;67:1653-1658

[92] Geraldes P, Sirois MG, Bernatchez PN, Tanguay JF. Estrogen regulation of endothelial and smooth muscle cell migration and proliferation: Role of p38 and p42/44 mitogen-activated protein kinase. Arteriosclerosis, Thrombosis, and Vascular Biology. 2002;22:1585-1590

[93] Spyridopoulos I, Sullivan AB, Kearney M, Isner JM, Losordo DW. Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Estradiol as a survival factor. Circulation. 1997;95:1505-1514

[94] Suzuma I, Mandai M, Takagi H, Suzuma K, Otani A, Oh H, et al. 17 beta-estradiol increases VEGF receptor-2 and promotes DNA synthesis in retinal microvascular endothelial cells. Investigative Ophthalmology & Visual Science. 1999;40:2122-2129

[95] Farhat MY, Vargas R, Dingaan B, Ramwell PW. In vitro effect of oestradiol on thymidine uptake in pulmonary vascular smooth muscle cell: Role of the endothelium. British Journal of Pharmacology. 1992;107:679-683

[96] Tofovic SP. Estrogens and development of pulmonary hypertension: Interaction of estradiol metabolism and pulmonary vascular disease. Journal of Cardiovascular Pharmacology. 2010;56:696-708. DOI: 10.1097/FJC.0b013e3181f9ea8d

[97] Semenza GL. HIF-1 and human disease: One highly involved factor. Genes & Development. 2000;14:1983-1991

[98] Semenza GL. HIF-1: Mediator of physiological and pathophysiological responses to hypoxia. Journal of Applied Physiology. 2000;88:1474-1480. DOI: 10.1152/jappl.2000.88.4.1474

[99] Lai YL, Law TC. Chronic hypoxia- and monocrotaline-induced elevation of hypoxia-inducible factor-1 alpha levels and pulmonary hypertension. Journal of Biomedical Science. 2004;11:315-321. DOI: 10.1159/000077099

[100] Liu W, Zhang Y, Lu L, Wang L, Chen M, Hu T. Expression and correlation of hypoxia-inducible factor-1alpha (HIF-1alpha) with pulmonary artery remodeling and right ventricular hypertrophy in experimental pulmonary embolism. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2017;23:2083-2088

[101] Fitzgerald G, Soro-Arnaiz I, De Bock K. The Warburg effect in endothelial cells and its potential as an anti-angiogenic target in cancer. Frontiers in Cell and Developmental Biology. 2018;6:100. DOI: 10.3389/fcell.2018.00100

[102] Basini G, Grasselli F, Bussolati S, Baioni L, Bianchi F, Musci M, et al. Hypoxia stimulates the production of the angiogenesis inhibitor 2-methoxyestradiol by swine granulosa cells. Steroids. 2011;76:1433-1436. DOI: 10.1016/j.steroids.2011.07.012
2-Methoxyestradiol in Pulmonary Arterial Hypertension: A New Disease Modifier
DOI: http://dx.doi.org/10.5772/intechopen.86812

[103] Docherty CK, Nilsen M, MacLean MR. Influence of 2-methoxyestradiol and sex on hypoxia-induced pulmonary hypertension and hypoxia-inducible factor-1-alpha. Journal of the American Heart Association. 2019;8:e011628, 10.1161/JAHA.118.011628

[104] Hao S, Jiang L, Fu C, Wu X, Liu Z, Song J, et al. 2-methoxyestradiol attenuates chronic-intermittent-hypoxia-induced pulmonary hypertension through regulating microRNA-223. Journal of Cellular Physiology. 2019;234(5):6324-6335. DOI: 10.1002/jcp.27363

[105] Tofovic SPRO, Jackson EK, Champion H, Schneider F. Estrogens exacerbates development of occlusive pulmonary arterial hypertension and formation of plexiform lesions. American Journal of Respiratory and Critical Care Medicine. 2012;185:A6803

[106] Tofovic SP, Bilan V, Jackson EK, Schneider F. Sugene 5416 dose-hypoxia-normoxia-gender interaction in angioproliferative pulmonary hypertension in rats. American Journal of Respiratory and Critical Care Medicine. 2014;189:A5566

[107] Tofovic SP, Rafikova O. Preventive and therapeutic effects of 2-methoxyestradiol, but not estradiol, in severe occlusive pulmonary arterial hypertension in female rats. American Journal of Respiratory and Critical Care Medicine. 2009;179:A1802

[108] Chan SY, Rubin LJ. Metabolic dysfunction in pulmonary hypertension: From basic science to clinical practice. European Respiratory Review: An Official Journal of the European Respiratory Society. 2017;26. DOI: 10.1183/16000617.0094-2017

[109] Tuder RM, Davis LA, Graham BB. Targeting energetic metabolism: A new frontier in the pathogenesis and treatment of pulmonary hypertension.

American Journal of Respiratory and Critical Care Medicine. 2012;185:260-266. DOI: 10.1164/rccm.201108-1536PP

[110] Li B, Yan J, Shen Y, Liu Y, Ma Z. Dichloroacetate prevents but not reverses the formation of neointimal lesions in a rat model of severe pulmonary arterial hypertension. Molecular Medicine Reports. 2014;10:2144-2152. DOI: 10.3892/mmr.2014.2432

[111] McMurtry MS, Bonnet S, Wu X, Dyck JR, Haromy A, Hashimoto K, et al. Dichloroacetate prevents and reverses pulmonary hypertension by inducing pulmonary artery smooth muscle cell apoptosis. Circulation Research. 2004;95:830-840. DOI: 10.1161/01.RES.0000145360.16770.9f

[112] Michelakis ED, McMurtry MS, Wu XC, Dyck JR, Moudgil R, Hopkins TA, et al. Dichloroacetate, a metabolic modulator, prevents and reverses chronic hypoxic pulmonary hypertension in rats: Role of increased expression and activity of voltage-gated potassium channels. Circulation. 2002;105:244-250

[113] Gorska-Ponikowska M, Kuban-Jankowska A, Daca A, Nussberger S. 2-methoxyestradiol reverses the pro-carcinogenic effect of L-lactate in osteosarcoma 143B cells. Cancer Genomics & Proteomics. 2017;14:483-493. DOI: 10.21873/cgp.20058

[114] Gorska-Ponikowska M, Kuban-Jankowska A, Eisler SA, Perricone U, Lo Bosco G, Barone G, et al. 2-methoxyestradiol affects mitochondrial biogenesis pathway and succinate dehydrogenase complex flavoprotein subunit a in osteosarcoma Cancer cells. Cancer Genomics & Proteomics. 2018;15:73-89. DOI: 10.21873/cgp.20067

[115] Zhao H, Jiang H, Li Z, Zhuang Y, Liu Y, Zhou S, et al. 2-methoxyestradiol
enhances radiosensitivity in radioresistant melanoma MDA-MB-435R cells by regulating glycolysis via HIF-1-alpha/PDK1 axis. International Journal of Oncology. 2017;50:1531-1540. DOI: 10.3892/ijo.2017.3924

[116] Osman MS, Michelakis ED. Immunity comes to play in the “sex paradox” of pulmonary arterial hypertension. Circulation Research. 2018;122:1635-1637. DOI: 10.1161/CIRCRESAHA.118.313075

[117] Batton KA, Austin CO, Bruno KA, Burger CD, Shapiro BP, Fairweather D. Sex differences in pulmonary arterial hypertension: Role of infection and autoimmunity in the pathogenesis of disease. Biology of Sex Differences. 2018;9:15. DOI: 10.1186/s13293-018-0176-8

[118] Sweatt AJ, Hedlin HK, Balasubramanian V, Hsi A, Blum LK, Robinson WH, et al. Discovery of distinct immune phenotypes using machine learning in pulmonary arterial hypertension. Circulation Research. 2019;124:904-919. DOI: 10.1161/CIRCRESAHA.118.313911

[119] Berstein LM, Larionov AA, Poroshina TE, Zimarina TS, Leenman EE. Aromatase (CYP19) expression in tumor-infiltrating lymphocytes and blood mononuclears. Journal of Cancer Research and Clinical Oncology. 2002;128:173-176. DOI: 10.1007/s00432-002-0322-9

[120] Hennebold JD, Daynes RA. Regulation of macrophage dehydroepiandrosterone sulfate metabolism by inflammatory cytokines. Endocrinology. 1994;135:67-75. DOI: 10.1210/endo.135.1.8013393

[121] Schmidt M, Kreutz M, Loffler G, Scholmerich J, Straub RH. Conversion of dehydroepiandrosterone to downstream steroid hormones in macrophages. The Journal of Endocrinology. 2000;164:161-169

[122] Rubinow KB. An intracrine view of sex steroids, immunity, and metabolic regulation. Molecular Metabolism. 2018;15:92-103. DOI: 10.1016/j.molmet.2018.03.001

[123] Shand FH, Langenbach SY, Keenan CR, Ma SP, Wheaton BJ, Schuliga MJ, et al. In vitro and in vivo evidence for anti-inflammatory properties of 2-methoxyestradiol. The Journal of Pharmacology and Experimental Therapeutics. 2011;336:962-972. DOI: 10.1124/jpet.110.174854

[124] Sutherland TE, Anderson RL, Hughes RA, Altmann E, Schuliga M, Ziogas J, et al. 2-methoxyestradiol—A unique blend of activities generating a new class of anti-tumour/anti-inflammatory agents. Drug Discovery Today. 2007;12:577-584. DOI: 10.1016/j.drudis.2007.05.005

[125] Tofovic SP, Salah EM, Dubey RK, Melhem MF, Jackson EK. Estradiol metabolites attenuate renal and cardiovascular injury induced by chronic nitric oxide synthase inhibition. Journal of Cardiovascular Pharmacology. 2005;46:25-35

[126] Zhang X, Jia Y, Jackson EK, Tofovic SP. 2-methoxyestradiol and 2-ethoxyestradiol retard the progression of renal disease in aged, obese, diabetic ZSF1 rats. Journal of Cardiovascular Pharmacology. 2007;49:56-63. DOI: 10.1097/FJC.0b013e31802cb88e

[127] Tofovic SP, Jones T, Petrusevska G. Dose-dependent therapeutic effects of 2-methoxyestradiol on monocrotaline-induced pulmonary hypertension and vascular remodelling. Prilozi. 2010;31:279-295

[128] Tofovic SP, Jones T, Bilan VP, Jackson EK, Petrusevska G. Synergistic therapeutic effects of 2-methoxyestradiol with either sildenafil or bosentan on amelioration of monocrotaline-induced pulmonary
hypertension and vascular remodeling. Journal of Cardiovascular Pharmacology. 2010;56:475-483. DOI: 10.1097/FJC.0b013e3181f215e7

[129] Tofovic SP, Salah EM, Mady HH, Jackson EK, Melhem MF. Estradiol metabolites attenuate monocrotaline-induced pulmonary hypertension in rats. Journal of Cardiovascular Pharmacology. 2005;46:430-437

[130] Alanko J, Sievi E, Lahteenmaki T, Mucha I, Vapaatalo H, Parantainen J. Catechol estrogens as inhibitors of leukotriene synthesis. Biochemical Pharmacology. 1998;55:101-104

[131] Tian W, Jiang X, Tamasioiuniene R, Sung YK, Qian J, Dhillon G, et al. Blocking macrophage leukotriene b4 prevents endothelial injury and reverses pulmonary hypertension. Science Translational Medicine. 2013;5:200ra117. DOI: 10.1126/scitranslmed.3006674

[132] Duncan GS, Brenner D, Tusche MW, Brustle A, Knobbe CB, Elia AJ, et al. 2-Methoxyestradiol inhibits experimental autoimmune encephalomyelitis through suppression of immune cell activation. Proceedings of the National Academy of Sciences of the United States of America. 2012;109:21034-21039. DOI: 10.1073/pnas.1215581110

[133] Luc JG, Paulin R, Zhao JY, Freed DH, Michelakis ED, Nagendran J. 2-Methoxyestradiol: A hormonal metabolite modulates stimulated T-cells function and proliferation. Transplantation Proceedings. 2015;47:2057-2066. DOI: 10.1016/j.transproceed.2015.05.021

[134] Xu L, Yang T, Su S, Wang F. 2-methoxyestradiol alleviates experimental autoimmune uveitis by inhibiting lymphocytes proliferation and T cell differentiation. BioMed Research International. 2016;2016:7948345. DOI: 10.1155/2016/7948345

[135] Zhou X, Liu C, Lu J, Zhu L, Li M. 2-Methoxyestradiol inhibits hypoxia-induced scleroderma fibroblast collagen synthesis by phosphatidylinositol 3-kinase/Akt/mTOR signalling. Rheumatology (Oxford, England). 2018;57:1675-1684. DOI: 10.1093/rheumatology/key166

[136] de Man FS, Tu L, Handoko ML, Rain S, Ruiter G, Francois C, et al. Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. American Journal of Respiratory and Critical Care Medicine. 2012;186:780-789. DOI: 10.1164/rccm.201203-0411OC

[137] Morrell NW, Stenmark KR. The renin-angiotensin system in pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine. 2013;187:1138-1139. DOI: 10.1164/rccm.201210-1872LE

[138] Morrell NW, Atochina EN, Morris KG, Danilov SM, Stenmark KR. Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. The Journal of Clinical Investigation. 1995;96:1823-1833. DOI: 10.1172/JCI18228

[139] Morrell NW, Danilov SM, Satyan KB, Morris KG, Stenmark KR. Right ventricular angiotensin converting enzyme activity and expression is increased during hypoxic pulmonary hypertension. Cardiovascular Research. 1997;34:393-403

[140] Morrell NW, Morris KG, Stenmark KR. Role of angiotensin-converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. The American Journal of Physiology. 1995;269:H1186-H1194. DOI: 10.1152/ajpheart.1995.269.4.H1186

[141] Morrell NW, Upton PD, Kotecha S, Huntley A, Yacoub MH, Polak JM, et al.
Angiotensin II activates MAPK and stimulates growth of human pulmonary artery smooth muscle via AT1 receptors. The American Journal of Physiology. 1999;277:L440-L448. DOI: 10.1152/ajplung.1999.277.3.L440

[142] Orte C, Polak JM, Haworth SG, Yacoub MH, Morrell NW. Expression of pulmonary vascular angiotensin-converting enzyme in primary and secondary plexiform pulmonary hypertension. The Journal of Pathology. 2000;192:379-384. DOI: 10.1002/1096-9896(2000)9999:9999<::AID-PATH715>3.0.CO;2-Q

[143] Ahn BH, Park HK, Cho HG, Lee HA, Lee YM, Yang EK, et al. Estrogen and enalapril attenuate the development of right ventricular hypertrophy induced by monocrotaline in ovariectomized rats. Journal of Korean Medical Science. 2003;18:641-648. DOI: 10.3346/jkms.2003.18.5.641

[144] Koganti S, Snyder R, Gumaste U, Karamyan VT, Thekkumkara T. 2-methoxyestradiol binding of GPR30 down-regulates angiotensin AT(1) receptor. European Journal of Pharmacology. 2014;723:131-140. DOI: 10.1016/j.ejphar.2013.10.064

[145] Koganti S, Snyder R, Thekkumkara T. Pharmacologic effects of 2-methoxyestradiol on angiotensin type 1 receptor down-regulation in rat liver epithelial and aortic smooth muscle cells. Gender Medicine. 2012;9:76-93. DOI: 10.1016/j.gendmed.2012.01.008

[146] Ogola B, Zhang Y, Iyer L, Thekkumkara T. 2-Methoxyestradiol causes matrix metalloproteinase 9-mediated transactivation of epidermal growth factor receptor and angiotensin type 1 receptor downregulation in rat aortic smooth muscle cells. American Journal of Physiology Cell Physiology. 2018;314:C554-C568. DOI: 10.1152/ajpcell.00152.2017

[147] Pingili AK, Davidge KN, Thirunavukkarasu S, Khan NS, Katsurada A, Majid DSA, et al. 2-methoxyestradiol reduces angiotensin II-induced hypertension and renal dysfunction in ovariectomized female and intact male mice. Hypertension. 2017;69:1104-1112. DOI: 10.1161/HYPERTENSIONAHA.117.09175

[148] Salah E, Bastacky SI, Jackson EK, Tofovic SP. 2-Methoxyestradiol attenuates angiotensin II-induced hypertension, cardiovascular remodeling, and renal injury. Journal of Cardiovascular Pharmacology. 2019;73:165-177. DOI: 10.1097/FJC.0000000000000649

[149] Assad TR, Hemnes AR. Metabolic dysfunction in pulmonary arterial hypertension. Current Hypertension Reports. 2015;17:20. DOI: 10.1007/s11906-014-0524-y

[150] Naderi N, Boobejame P, Bakhshandeh H, Amin A, Taghavi S, Maleki M. Insulin resistance in pulmonary arterial hypertension, is it a novel disease modifier? Research in Cardiovascular Medicine. 2014;3:e19710. DOI: 10.5812/cardiovascmed.19710

[151] Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, et al. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. Circulation. 2007;115:1275-1284. DOI: 10.1161/CIRCULATIONAHA.106.663120

[152] Summer R, Walsh K, Medoff BD. Obesity and pulmonary arterial hypertension: Is adiponectin the molecular link between these conditions? Pulmonary Circulation. 2011;1:440-447. DOI: 10.4103/2045-8932.93542

[153] Umar S, Partow-Navid R, Ruffenach G, Iorga A, Moazeni S,
Eghbali M. Severe pulmonary hypertension in aging female apolipoprotein E-deficient mice is rescued by estrogen replacement therapy. Biology of Sex Differences. 2017;8:9. DOI: 10.1186/s13293-017-0129-7

[154] Annerbrink K, Westberg L, Nilsson S, Rosmond R, Holm G, Eriksson E. Catechol O-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. Metabolism: Clinical and Experimental. 2008;57:708-711. DOI: 10.1016/j.metabol.2008.01.012

[155] Hall KT, Jablonski KA, Chen L, Harden M, Tolkin BR, Kaptchuk TJ, et al. Catechol-O-methyltransferase association with hemoglobin A1c. Metabolism: Clinical and Experimental. 2016;65:961-967. DOI: 10.1016/j.metabol.2016.04.001

[156] Barchiesi F, Jackson EK, Fingerle J, Gillespie DG, Odermatt B, Dubey RK. 2-methoxyestradiol, an estradiol metabolite, inhibits neointima formation and smooth muscle cell growth via double blockade of the cell cycle. Circulation Research. 2006;99:266-274. DOI: 10.1161/01.RES.0000233318.85181.2e

[157] Tofovic SP, Hu J, Jackson EK. Schneider F 2-hydroxyestradiol attenuates metabolic syndrome-induced pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine. 2015;191:A4096

[158] Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. The New England Journal of Medicine. 1993;328:1732-1739. DOI: 10.1056/NEJM199306173282402

[160] Tuder RM, Cool CD, Geraci MW, Wang J, Abman SH, Wright L, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine. 1999;159:1925-1932. DOI: 10.1164/ajrccm.159.6.9804054

[159] Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. The New England Journal of Medicine. 1993;328:1732-1739. DOI: 10.1056/NEJM199306173282402

[161] Dubey RK, Jackson EK, Keller PJ, Imthurn B, Rosselli M. Estradiol metabolites inhibit endothelin synthesis by an estrogen receptor-independent mechanism. Hypertension. 2001;37:640-644

[162] Hill BJ, Gebre S, Schlicker B, Jordan R, Necessary S. Nongenomic inhibition of coronary constriction by 17ßs-estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol. Canadian Journal of Physiology and Pharmacology. 2010;88:147-152. DOI: 10.1139/Y09-120

[163] Seeger H, Mueck AO, Lippert TH. Effect of estradiol metabolites on prostacyclin synthesis in human endothelial cell cultures. Life Sciences. 1999;65:PL167-PL170

[164] Tsukamoto A, Kaneko Y, Yoshida T, Han K, Ichinose M, Kimura S. 2-Methoxyestradiol, an endogenous metabolite of estrogen, enhances apoptosis and beta-galactosidase expression in vascular endothelial cells. Biochemical and Biophysical Research Communications. 1998;248:9-12. DOI: 10.1006/bbrc.1998.8902

[165] Fenoy FJ, Hernandez ME, Hernandez M, Quesada T, Salom MG, Hernandez I. Acute effects of 2-methoxyestradiol on endothelial aortic NO release in male and ovariectomized female rats. Nitric oxide:
Biology and Chemistry. 2010;23:12-19. DOI: 10.1016/j.niox.2010.03.002

[166] Chen W, Cui Y, Zheng S, Huang J, Li P, Simoncini T, et al. 2-methoxyestradiol induces vasodilation by stimulating NO release via PPARgamma/PI3K/Akt pathway. PLoS One. 2015;10:e0118902. DOI: 10.1371/journal.pone.0118902

[167] Landeros RV, Pastore MB, Magness RR. Effects of the catechol and methoxy metabolites of 17beta-estradiol on nitric oxide production by ovine uterine artery endothelial cells. Reproductive Sciences. 2019;26:459-468. DOI: 10.1177/1933719118783265

[168] Berg D, Sonsalla R, Kuss E. Concentrations of 2-methoxyoestrogens in human serum measured by a heterologous immunoassay with an 125I-labelled ligand. Acta Endocrinologica. 1983;103:282-288

[169] Tofovic SP, Jackson EK, Piche C. Pharmacokinetics and safety of a subcutaneously injected long-acting formulation of 2-methoxyestradiol (2ME) in healthy volunteers. Basic & Clinical Pharmacology & Toxicology. 2009;105:107