



Inhibiting oestrogen signalling in pulmonary arterial hypertension: sex, drugs and research

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Inhibition of oestrogen signalling in PAH is currently being studied clinically but several questions remain <http://ow.ly/G7xK30cPN5M>

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Pulmonary arterial hypertension (PAH) is a sexually dimorphic disease that affects women more than men. Several pre-clinical and clinical studies identified 17 β -oestradiol (E2), the most abundant female sex hormone, and/or its metabolite 16 α -hydroxyoestrone as disease mediators in PAH (reviewed by LAHM *et al.* [1], and FODERARO and VENTEUOLO [2]). A recent case-control study even demonstrated significantly higher levels of E2 in men with PAH compared to controls [3].

E2 is mostly synthesised through aromatisation of androgens by the enzyme aromatase (encoded by the *CYP19A1* gene). In females, this process primarily occurs in the ovaries, though later in life, extragonadal E2 production (e.g. in adipocytes) becomes the predominant source (as it is in males throughout life). Classical E2 signalling is characterised by activation of the oestrogen receptors ER α and/or ER β , which translocate to the nucleus and act as transcription factors by binding to oestrogen response elements of target genes (reviewed by MURPHY [4]). However, several variations of this pathway exist, including nongenomic signalling and signalling through an orphan G-protein coupled receptor [1, 4]. Aromatase and oestrogen receptors are found in both sexes and in every major organ system (including the lung and the right ventricle [5–8]), implying that oestrogens can have biologically relevant effects throughout the body.

Preclinical and clinical studies implicate aromatase as a potential contributor to PAH pathogenesis [6, 9]. Similarly, oestrogen receptor-mediated signalling has been linked to PAH development [8]. These studies have led to a recently published, small phase II randomised clinical trial (RCT) of aromatase inhibition with anastrozole in postmenopausal women and men with PAH [10]. This study demonstrated that anastrozole decreased serum E2 levels by 40% and increased 6-min walk distance, leading the authors to conclude that anastrozole therapy appeared safe and warranted further evaluation. Based on these results, a

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larger, multicentre phase II RCT has been funded by the National Heart, Lung, and Blood Institute (Pulmonary Hypertension and Anastrozole Trial (PHANTOM)) and a smaller pilot trial of fulvestrant (a nonselective oestrogen receptor antagonist) is ongoing (www.clinicaltrials.gov identifier number NCT02911844).

This issue of the *European Respiratory Journal* includes a report of a study by CHEN *et al.* [11] that provides further support for the promise of oestrogen inhibition as a therapeutic strategy in PAH. Using bone morphogenetic protein II (*Bmpr2*)-mutant mice, the authors show that administration of anastrozole plus fulvestrant reduced right ventricular systolic pressures and pulmonary artery remodelling, and increased expression of peroxisome proliferator-activated receptor (PPAR)- γ and CD36 (also known as fatty acid translocase). Since the anastrozole/fulvestrant combination also attenuated increases in HOMA-IR (a marker of insulin resistance [12]) seen in *Bmpr2* mutants, the authors interpreted the increase in PPAR- γ and CD36 as a sign of decreased insulin resistance. In addition, by crossing *Bmpr2*-mutant mice with mice lacking either *Esr1* (the gene encoding ER α) or *Esr2* (encoding ER β), the authors elegantly demonstrated that both receptors may contribute to pulmonary hypertension development in *Bmpr2* mutants, with the effects of *Esr2* knockout being more robust (suggesting a stronger contribution of ER β). Furthermore, the authors showed that treatment with the selective oestrogen receptor modulator tamoxifen has a moderate inhibitory effect on pulmonary artery remodelling (but not on right ventricular systolic pressure).

Strengths of the study include the relevant genetic animal model that spontaneously develops pulmonary hypertension, the sophisticated *Bmpr2-Esr* crossing experiments, the use of a treatment as well as a prevention approach, and the use of anastrozole, fulvestrant and tamoxifen in doses similar to those used in human studies. The latter two drugs are of particular interest, since they have not been extensively studied in animal models of PAH. The data presented are important in their support of the relevance of current and future clinical trials targeting interventions on oestrogen signalling pathways. In addition, the study further solidifies the potential link between oestrogens and metabolic alterations in PAH [13].

However, the study also has several important weaknesses. First, despite the direct relevance to patients (using a *Bmpr2* mutation), this experimental model of PAH is known to be mild. For example, right ventricular systolic pressures were only moderately increased and cardiac output was preserved in the *Bmpr2* mutants, whereas patients with PAH typically exhibit significant increases in pulmonary artery pressures and markedly decreased cardiac outputs at presentation [14–16]. Second, anastrozole and fulvestrant were given in combination and were not studied independently. It is therefore unknown whether this combination has additive or synergistic effects compared to the use of either drug alone. This is important, since each drug has side-effects and fulvestrant is expensive. While adequate suppression of oestrogen receptor signalling with fulvestrant was assumed in the current study based on the dose used, this was not experimentally confirmed. Third, the *Esr1* knockout model used is not considered a complete knockout (due to the presence of an ER α splice variant [17]); this may explain the finding that the effects of *Esr2* knockout were more robust, while other investigators have found ER α to be pathophysiologically relevant [8]. However, differences in models and cell type-specific effects may contribute to this discrepancy as well. Lastly, the current study demonstrated detrimental effects of high-dose 16 α -hydroxyoestrone on mitochondrial function, but did not elucidate whether the negative effects were mediated by E2 or rather by 16 α -hydroxyoestrone, a question that has important clinical implications when identifying the optimal strategy to inhibit oestrogenic signalling.

What do the currently available data suggest when it comes to clinical strategies aimed at inhibiting oestrogenic signalling in PAH? Based on the results of the pilot human trial [10] and recent experimental studies in a model of more severe PAH [6], a phase II study of anastrozole is currently underway. Could other strategies for inhibiting oestrogenic signalling be beneficial? For example, if 16 α -hydroxyoestrone is indeed the culprit, specifically inhibiting the conversion of E2 to this metabolite could be promising, as previously shown in an animal study [7]. Alternatively, fulvestrant may be more efficacious than aromatase inhibition [18, 19]. Lastly, a combination approach as pursued by CHEN *et al.* [11] may result in the most profound anti-oestrogenic effects. However, the most dramatic suppression of oestrogenic signalling could also have the most side-effects, such as depression, osteoporosis and loss of muscle mass [20], decreasing tolerance and adherence. PHANTOM does not include premenopausal women (since anastrozole does not suppress E2 production with ovarian function); CHEN *et al.* [11] speculate that tamoxifen may be an alternative to inhibit oestrogenic signalling in premenopausal women, but more study is needed.

An issue of major importance is the possibility that oestrogens may not be uniformly detrimental in PAH. Women, while more prone to developing PAH, exhibit better survival than their male counterparts [14–16]. This finding is accompanied by more favourable haemodynamics and better right ventricular function in female patients [15, 21–23]. A role of E2 in mediating these effects is suggested by both

preclinical studies and clinical observations [5, 24, 25]. These studies suggest direct effects of E2 on the right ventricle, where it stimulates antiapoptotic, anti-inflammatory and proangiogenic signalling, as well as indirect right ventricle-protective effects mediated by inhibition of collagen accumulation in the proximal pulmonary artery [5, 24–27]. In addition, E2's pulmonary artery vasodilator effects may help unload the right ventricle [28]. Further evidence for a role of oestrogens in modulating right ventricular function comes from a recent study demonstrating that polymorphisms in oestrogen-metabolising enzymes affect right ventricular function [29]. Oestrogen inhibition therefore has the potential to adversely affect the right ventricle, although the broad use of these medications in the general population to treat breast cancer has never generated a post-marketing signal of right ventricular dysfunction. Larger placebo-controlled trials will be necessary and may identify populations that are most likely to benefit from oestrogen inhibition (*e.g.* patients with early disease).

Lastly, it is worthwhile to point out that E2, its metabolites and its receptors exert a variety of complex and sometimes even contradictory effects, sometimes even within the same organ system [4, 30]. This reflects the fact that net oestrogen effects are affected by multiple factors, such as oestrogen receptor abundance and distribution, presence or absence of co-activators or co-inhibitors, receptor activation by non-oestrogen ligands and activity of metabolising enzymes [4, 30]. All these variables are affected by sex, age, nutrition, environmental exposures and other variables [4, 30]. Oestrogen effects are therefore highly context and compartment specific. For example, E2 has been shown to stimulate pulmonary artery smooth muscle cell proliferation but to exert anti-proliferative effects in pulmonary artery endothelial cells [6–8, 31]. Similarly, in a recent microarray analysis of lungs from chronically hypoxic rats treated with E2, the E2-regulated genome indicated that E2 regulates multiple genes whose activation or inhibition is consistent with anti-proliferative effects, but that E2 also induces changes consistent with pro-proliferative or pro-angiogenic effects [32].

These complexities indicate that we have to carefully dissect oestrogenic pathways in order to get a detailed understanding of oestrogen's biological effects in the pulmonary vasculature and right ventricle in health and disease. It is possible that oestrogen-directed therapies will need to be employed in a “precision medicine” approach, guided by age, sex, baseline right ventricular function, presence of menopause, genetic variants, measurement of E2 levels, or quantification of lung or right ventricular oestrogen receptor expression [33]. The study by CHEN *et al.* [11] further advances the field and highlights the work that needs to be done. Only an informed and focused approach will allow us to effectively and safely target oestrogen signalling in PAH.

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