The biology of pulmonary arterial hypertension (PAH) is full of mysteries, and one of its longer-standing ones has also intrigued and inspired both scientists and artists throughout history: the female sex. Although affecting patients of all ages and both sexes, PAH preferentially affects young women, suggesting that the female sex is a risk factor for PAH. Even in registries (the National Institutes of Health registry in the 80s,2 the French registry,3 and the Registry to Evaluate Early and Long-term PAH Disease Management (REVEAL),4 respectively). On the other hand, the disease can be more severe in men. Female animals with PAH tend to have lower pulmonary artery pressures and better outcomes compared with males,5 and similarly, male PAH patients have higher mortality than females.6 The basis for this apparent paradox remains unknown.

Estrogens exert their effects through both genomic effects, by activating their cytosolic or membrane-bound receptors, and nongenomic effects, by activating membrane G protein–coupled estrogen receptors. For example, via the nongenomic effects, estrogens promote the release of both prostacyclin and nitric oxide while, via the genomic effects, they decrease the expression of endothelin 1 under hypoxia, all promoting vasoconstriction in the pulmonary circulation.5 Yet, the pathology of PAH is driven not by vasoconstriction, but rather inflammatory, promitogenic, and proangiogenic effects.5,11,12 On the other hand, the 2-OH-estradiol metabolites have shown antiproliferative and antiangiogenic effects and, when given exogenously in animal models of PAH, have shown evidence of disease regression. For example, 2-MeO-estradiol (2-MeO-estradiol) is formed rapidly, a metabolite that has received attention for its potential therapeutic role in many diseases. When CYP1B1 is inhibited, metabolism can be shifted to the hydroxylation at C16 forming 16α-OH-estradiol and 16α-OH-estrone, metabolites that exhibit pro-inflammatory, promitogenic, and proangiogenic effects.5,11,12

Thus, individuals who metabolize a larger proportion of estrogen to 16α-OH-estradiol and 16α-OH-estrone, compared with 2-OH-estradiol, may be at increased risk for proliferative and inflammatory diseases, and this risk can be potentially assessed by measuring urinary 2-OH-estradiol/16α-OH-estrone ratio. Because CYP1B1 is the most efficient estradiol hydroxylase,5,10 the 2-OH-estradiol/16α-OH-estrone ratio is an indicator of CYP1B1 activity. CYP1B1 is highly expressed in various human hormone-regulated cancers compared with normal tissues. However, despite early indications that polymorphisms in the CYP1B1 would result in enzymatic activity changes increasing cancer risk in hormone-sensitive tissues like the breast,17 recent meta-analyses have failed to support this concept.18 Nevertheless, this idea has also been explored in PAH.

In a study using Affymetrix arrays to compare asymptomatic BMPR2 mutation carriers (unaffected by PAH) to carriers affected by PAH, CYP1B1 expression was almost 10-fold decreased in affected female BMPR2 carriers.19 To address the background noise caused by environmental and drug-
be selective for the pulmonary arteries; for example, it did not take place in the right ventricle. They also showed that CYP1B1 is overexpressed in the pulmonary arteries of a few idiopathic PAH and heritable PAH, compared with non-PAH, human lungs. They then showed that deletion of the CYP1B1 gene in mice (CYP1B1−/−) attenuated the degree of hypoxia-induced PAH compared with wild-type (WT) male but not female mice. Female CYP1B1−/− mice developed PAH similarly to WT mice, with a similar increase in right ventricular systolic pressure and vascular remodeling. However, despite the same increase in pressure, the CYP1B1−/− mice had less right ventricular hypertrophy compared with the WT mice. Although the levels of right ventricular CYP1B1 did not change in the hypoxia-induced PAH model, it is possible that the CYP1B1−/− mice had different circulating estrogen metabolites that could affect the right ventricle directly. The dissociation between the increase in pressure and right ventricular hypertrophy is intriguing, but it cannot be interpreted fully because the authors did not measure the effects of estrogens on the right ventricle ex vivo and did not measure mean pulmonary artery pressure and cardiac output to report pulmonary vascular resistance. For example, a decrease in right ventricular systolic pressure may reflect a primary decrease in right ventricular contractility and not a decrease in right ventricular afterload. The authors did not measure estrogen metabolite levels in these mice, limiting our ability to extrapolate to PAH patients with decreased CYP1B1 levels and 2-OH-estradiol/16α-OH-estrone ratios. Nevertheless, although the results of the current study appear in conflict with the human genetic CYP1B1 studies, it is important to remember that in the former, expression of protein in the pulmonary arteries is measured whereas in the latter gene expression in immortalized B cells was reported.

The authors also confirmed convincingly that the proliferative effects of 16α-OH-estrone in vitro and in vivo are promoting PAH. Administration of 16α-OH-estrone during 28 consecutive days in mice induced an increase in right ventricular systolic pressure, pulmonary vascular remodeling, and right ventricular hypertrophy, compared with vehicle-treated mice. They then showed that 2,3′,4,5′-tetramethoxystilbene (TMS) was beneficial in 2 models of mice PAH under disease prevention protocols. Although the effects were modest, they were convincing. However, although TMS is frequently used as a CYP1B1 inhibitor, it is difficult to interpret its effects based on CYP1B1 inhibition. This is because the effects of the drug on CYP1B1 levels or activity in the pulmonary arteries of these mice were not measured. Moreover, it is difficult to understand how complete absence of an enzyme (as is the case in CYP1B1−/− mice) has less effect than a putative inhibitor of the enzyme. In other words, it is difficult to understand that although hypoxic female CYP1B1−/− mice had the same degree of pulmonary hypertension with the hypoxic female WT mice (Figure 3B of the White et al[21] article), TMS attenuated pulmonary hypertension in the same hypoxic female WT mice (Figure 4B of the White et al[21] article). This suggests that the drug has additional off-target effects. This possibility could have been addressed by showing absence of TMS effects on CYP1B1−/− mice.

TMS is a resveratrol analog (Figure 2B), a natural compound that activates the deacetylase Sirtuin 1, which has
received a lot of attention because of its involvement in the biology of aging. Although resveratrol also has multiple effects, by activating Sirtuin 1 it can regulate the activity of both members of the peroxisome proliferator-activated receptor gamma coactivator-1α/estrogen-related receptor complex, which are essential metabolic regulatory transcription factors. Although the estrogen-related receptors do not directly respond to 17β-estradiol, they can bind to estrogen response elements, thus regulating mitochondrial and metabolic functions. Indeed, estrogens have been shown to regulate multiple aspects of mitochondrial biology. Thus the possibility that TMS (like resveratrol) could have off-target effects on mitochondrial function has to be considered because it is now increasingly being recognized that metabolism and mitochondria may be central to PAH biology. In fact, resveratrol has been shown to prevent monocrotaline PAH in rats. In keeping with off-target non–CYP1B1-mediated effects of TMS are studies in cancer showing TMS-dependent inhibition of activated focal adhesion kinase (FAK), Akt, and mammalian target of rapamycin. In summary, TMS may have therapeutic value for PAH, but it is likely that this is not based on CYP1B1 inhibition alone.

The authors brought to our attention the CYP1B1−/− mice as a new model in which to study many aspects of the estrogen puzzle in PAH. Although it is not clear that CYP1B1 can be a therapeutic target in PAH, it may be an important disease modifier because of its ability to regulate local estrogen metabolism in the pulmonary arteries, and it may lead to biomarker discoveries. More work is needed to determine whether TMS is a potential therapy for PAH. It is important for a mechanism to be well characterized before experimental therapies can be translated to humans.

Future studies in this important field need to provide comprehensive data on levels of estrogen metabolites in animals or patients and provide careful comprehensive hemodynamic assessment of the pulmonary artery–right ventricle unit. The possibility that estrogens and their metabolites may have direct effects on the right ventricle has to be considered because it is the right ventricle that primarily drives morbidity and mortality in PAH. For example, as a result of higher estrogen levels, females may have more vulnerable pulmonary circulation for the development of PAH but stronger right ventricles. Indeed, both in PAH patients and healthy subjects, right ventricular ejection fraction is lower in males compared with females.

Until then, the estrogen mysteries will continue to puzzle us. At the end, if one of the brightest minds of our century, the cosmologist Stephen Hawking, still struggles to understand women, who are we to solve the estrogen puzzle in a disease already full of mysteries?

Figure 2. A, Left, Ratio of urinary 2-hydroxyoestrogen (2-OH-estradiol) to 16-hydroxyoestrone (16-OH-estrone) in female BMPR2 mutation carriers affected by PAH (AMCs) versus female BMPR2 mutation carriers unaffected by PAH (UMCs). AMCs have a significantly reduced ratio compared to UMCs (P=0.006). Right, This decrease in the ratio in the affected patients may be due to the fact they have a markedly decrease expression of CYP1B1 in their circulating B cells, compared to unaffected mutation carriers as discussed in the text. Reprinted with permission from West et al. Copyright SpringerOpen, 2008) and from Austin et al. (Copyright European Respiratory Society, 2009). B, The structure of TMS is impressively similar to the structure of Resveratrol (see text).
Disclosures

None.

References


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