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 heritable PAH associated with autosomal dominant mutations in the gene encoding the bone morphogenetic protein receptor type 2 (BMPR2), women after puberty are ≈2.5 times more likely to develop PAH than males. In idiopathic PAH the female/male ratio ranges from 1.7:1 or 1.9:1 to 4.1:1 in 3 published PAH registries (the National Institutes of Health registry in the 80s, the French registry, and the Registry to Evaluate Early and Long-term PAH Disease Management (REVEAL), 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, and similarly, male PAH patients have higher mortality than females. 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 vasoconstriction in the pulmonary circulation. Yet, the pathology of PAH is driven not by vasoconstriction, but rather by a proproliferative and antiapoptotic diathesis throughout the pulmonary arterial wall.

The main circulating estrogen in women, estradiol, has diverse effects in different vascular beds, but in pulmonary arterial smooth muscle cells in vitro it enhances proliferation in a dose-dependent manner. However, the same group showed that estradiol improved monocrotaline-induced PAH, suggesting that, in vivo, its effects are more complex. The complexity increases by the observation that the effects of estrogens often depend on the animal model in which they were tested.

Our understanding of the estrogen puzzle has improved by studies exploring the metabolites downstream of estrogens, by >15 human cytochrome P450 isoforms. Dozens of metabolites are produced, with variable and often opposing biological effects, particularly in terms of cell proliferation. The P450-based enzyme CYP1B1 has received attention for its role in many diseases, including cancer. It catalyzes principally the hydroxylation of estradiol at positions C2 and C4, forming 2-OH-estradiol and 4-OH-estradiol, the former being the dominant pathway. In the presence of the COPT (catechol-O-methyltransferase), 2-Methoxy-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 proinflammatory, promitogenic, and proangiogenic effects. 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 has antiproliferative effects in pulmonary arterial smooth muscle cells and fibroblasts, inhibits hypoxia inducible factor-1α and angiogenesis, and attenuates PAH in animal models.

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, 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, recent meta-analyses have failed to support this concept. 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. To address the background noise caused by environmental and drug-
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chronic hypoxia+SU5416 mice). This increase appeared to
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 hypox-
ia-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. How-
ever, 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 circu-
lating estrogen metabolites that could affect the right ventri-
cle 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
decline 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.

Figure 1. Estrogen metabolites: The P450-based enzyme
CYP1B1 is critical for the balance of good and bad metabolites
in terms of cellular proliferation, inflammation, or angiogenesis
and thus in their ability to promote proliferative and inflamma-
tory diseases like cancer or PAH (see text for details). PAH indi-
cates pulmonary arterial hypertension.

dependent factors, the investigators used patient-derived im-
mortalized circulating B cells, instead of pulmonary vascular
or other PAH-relevant tissues. These authors also showed a
higher frequency of a polymorphism on the CYP1B1 gene
(N453S) that has been associated with decreased expression
in cancer tissues. In a study of 140 BMPR2 mutation carriers
(86 females and 54 males), a 4-fold higher PAH penetrance
was found in females homozygous for the wild-type genotype
(N/N) than those with N/S or S/S genotypes.20 Moreover, in
a small subgroup of these patients, the 2-OH-estradiol/16α-
OH-estrone ratio was 2.3-fold lower in affected BMPR2
mutation female carriers compared with unaffected carriers20
(Figure 2A). Taken together, these results suggest that the
decreased level of CYP1B1 may explain the low ratio of
protective versus harming metabolites, which would promote
the development of PAH in female BMPR2 mutation carriers.
Although these authors did not study PAH patients without
BMPr2 mutations, extrapolation of their results would sug-
gest that mice lacking CYP1B1 may develop worse pulmo-
nary hypertension when exposed to a disease trigger like
hypoxia, as a result of a relative lack of the protective
metabolites like 2-MeO-estradiol.

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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 1-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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Roxane Paulin and Evangelos D. Michelakis

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