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Complexities of oestradiol pharmacology in pulmonary arterial hypertension

To the Editor:

We read with great interest the recent article by YUAN *et al.* [1]. The authors confirm previous observations that female sex and E_2 (17β-oestradiol) protect against a rat model of pulmonary hypertension (PH), namely monocrotaline-induced PH (MCT-PH), and that the beneficial effects of E_2 in MCT-PH are associated with increased lung nitric oxide, prostacyclin levels and reduced endothelin levels. Furthermore, based on E_2 plasma levels and altered expression of key enzymes, they suggest that E_2 deficiency may play a role in MCT-PH.

We would like to draw attention to another dimension of E_2 pharmacology that adds to the complexity of E_2 in experimental PH and human pulmonary arterial hypertension (PAH), *i.e.* the metabolism of E_2 to biologically active metabolites. We also would like to discuss the concept that the overall effects of sex and E_2 in PH may depend on the model system.

Despite the fact that E_2 and female sex protect against MCT-PH, surprisingly PAH occurs more frequently in females than males. This incongruous finding is called the ''PAH gender paradox'' [2]. Even so, females with PAH have better right ventricular function and survival compared to males with PAH [3–5]. The apparent contradictions posed by the effects of female sex and oestrogens in experimental PH *versus* human PAH could be explained by the complexity of E_2 metabolism, the opposing effects of E_2 *versus* its metabolites on endothelial vascular remodelling, limitations of the experimental models used, and the opposite roles that E_2 may play in the pulmonary vasculature *versus* the right ventricle.

Several lines of evidence strongly suggest that the vascular protective effects of E_2 are mediated largely by its downstream metabolites [2]. Notably, 2-methoxyestradiol (2ME, a major non-oestrogenic metabolite of E_2) attenuates the development of and retards the progression of MCT- or hypoxia-induced PH in male rats and mediates the protective effects of E_2 in female rats with MCT- or bleomycin-induced PH [2]. Furthermore, 2ME is decisively more potent than E_2 in increasing prostacyclin synthesis and release, in inhibiting endothelin synthesis in endothelial cells, in stimulating nitric oxide release from endothelium, and in inhibiting growth of systemic and pulmonary vascular smooth muscle cells [2]. Similar to the effects of E_2 in the YUAN *et al.* [1] study, inhibition of vascular remodelling by 2ME is also associated with down regulated expression of phosphorylated Akt and up regulated expression of cyclooxygenase-2 [6]. Thus, at least in part, the beneficial effects of oestradiol in MCT-PAH are mediated by 2ME.

The hallmark vascular change in MCT-PH is medial thickening and neomuscularisation, with only mild endothelial damage that does not lead to occlusive proliferation of endothelial cells and formation of plexiform lesions. E_2 and 2ME have opposing effects on endothelial cells: E_2 is promitogenic, proangiogenic and antiapoptotic, whereas 2ME is antimitogenic, antiangiogenic and proapoptotic. This may have significant ramifications for the overall effects of oestrogens in experimental PH. We suggested that the effects of sex and oestrogens in experimental PH should be studied in a model of severe angio-proliferative PH (administration of vascular endothelial growth factor 2 receptor antagonist SU5416 and exposure to hypoxia) [2]. This model, despite its own limitations, more closely mimics the key vascular alterations and progressive and deadly character of disease seen in severe PAH in humans. In a recent study in this model (and contrary to the effects of sex and E_2 in the study by YUAN *et al.* [1]), we demonstrated that female rats develop more severe PH than males (numerous plexiform lesions in females compared to predominantly occlusive lesions in male rats), that in ovariectomised rats rescue treatment with E_2 exacerbates vascular lesions and

PH, and that E_2 induces right ventricle–pulmonary vasculature uncoupling: despite more severe PAH, females had significantly reduced right ventricular hypertrophy, and ovariectomy exacerbated the increase in right ventricular mass, an effect that was reversed by exogenous E_2 [2, 7]. Therefore, MCT-PH may not be the best model for studying the complexities of oestrogen pharmacology in human PAH.

The concept that E_2 deficiency is involved in MCT-PH may be correct; but this concept may be incorrect in human PAH. Monocrotaline is a toxin that, in male rats, reduces plasma testosterone levels by 50%, and gonadal toxicity could be the reason for reduced E_2 levels. Furthermore, in the YUAN *et al.* [1] study, animals were not synchronised for oestrous cycle, and this may also have contributed to differences in plasma E_2 levels. It is noteworthy that recent clinical data suggest that female sex and increased, rather than decreased, E_2 levels and P450 Cyp19 (aromatase) activity are associated with greater risk of developing portopulmonary PAH [8].

Oestradiol may exert beneficial effects on the right ventricle, yet may exacerbate PAH and pulmonary vascular lesions. The question of whether oestradiol is good, bad, or both in human PAH requires further investigation. We view E_2 as a double-edged sword in human PAH.

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From the authors:

We thank S.P. Tofovic and E.K. Jackson for their interest in our study [1]. It is well known that pulmonary arterial hypertension (PAH) is a female-predominant disease [2–4], and this seems to indicate that oestrogens may be predisposing factors. However, this contrasts with the fact that females with PAH have preserved right ventricle function and survival compared with males with PAH [2–5], suggesting that oestrogens could be protective of PAH. So, oestrogens may be viewed as a double-edged sword in PAH. To explore the role of oestrogens in PAH, we observed the effect of endogenous and exogenous E_2 (17β-oestradiol) in rats with monocrotaline (MCT)-induced pulmonary hypertension (PH) [1]. Our results suggested similar protective roles of endogenous and exogenous E_2 in pulmonary vascular remodelling and right ventricular hypertrophy in MCT-induced PH rats, which is similar to the effect of E_2 reported in the study of UMAR *et al.* [6]. E_2 therapy has been shown to rescue both pulmonary vasculopathy and right ventricle function in rats with PH. Thus, our results do not support the opinion expressed by S.P.Tofovic and E.K. Jackson that E_2 may have opposite roles in the pulmonary vasculature and the right ventricle.

In the study described by S.P. Tofovic and E.K. Jackson, 2-methoxyoestradiol (2ME) attenuated the development of PH in rats and was decisively more potent than E_2 in increasing prostacyclin and nitric oxide release, inhibiting endothelin-1 synthesis in endothelial cells, and inhibiting growth of systemic and pulmonary vascular smooth muscle cells. We do not exclude the possibility that the beneficial effects of oestradiol in MCT-induced PH are mediated, at least in part, by 2ME. Therefore, it is a good suggestion by S.P. Tofovic and E.K. Jackson that the effects of E_2 and its metabolites are studied in other animal models of PH, which may further confirm the overall effects of E_2.

We have conducted a cohort study at our centre in which serum concentrations of oestradiol were measured in 120 adult fertile female patients with incident idiopathic PAH and 120 age- and sex-matched healthy female controls. Serum oestradiol concentrations were significantly decreased in PAH patients compared with controls (median (interquartile range): 184.6 (111.9–351.3) *versus* 271.4 (158.4–497.6) $pmol \cdot L^{-1}$; $p<0.001$). The same trend of was observed in serum oestradiol concentrations in 42 female patients with idiopathic PAH and 42 female controls studied in the follicular phase (median (interquartile range): 166.2 (114.1–223.6) *versus* 195.4 (144.2–287.7) $pmol \cdot L^{-1}$; $p=0.03$) (fig. 1). The data indicated that serum oestradiol concentrations were