Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue3abst023P.pdf

Evidence of a Pathogenic Oestrogen/Serotonin Axis in Pulmonary Hypertension

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Introduction 17β oestradiol (E2) and its metabolites are emerging as highly pathogenic mediators in various diseases including pulmonary hypertension (PH): a devastating vasculopathy of the pulmonary arteries. In particular, we have recently provided unique evidence that E2 metabolism by cytochrome P450 1B1 (CYP1B1) is pathogenic in both experimental PH and human PAH[1]. CYP enzymes hydroxylate oestrogens to the 2-, 4- and 16-hydroxyoestrogens (2-OHE, 4-OHE and 16-OHE, respectively). The catechol oestrogens can undergo further metabolism to the respective methoxyoestrogens by catechol-O methyltransferase (COMT). Overexpression of the serotonin transporter (SERT) in mice (SERT+ mice) leads to a spontaneous PH phenotype in females only; an effect that is reversed by ovariectomy[2]. Thus, we hypothesized that the E2 metabolic axis is pathologically altered in female SERT+ mice.

Methods Protein analysis for aromatase, the E2 synthesizing enzyme and CYP1B1 was determined by western blotting in whole lung homogenates from wildtype (C57B/6JXCBA) and SERT+ mice. E2 levels were determined in these samples by ELISA (Cayman Chemicals). To assess the therapeutic potential of a CYP1B1 inhibitor, SERT+ mice were dosed with the selective inhibitor 2,3',4,5'-Tetramethoxystilbene (TMS, Tocris; 1.5mg/kg/day i.p. for 14 days; vehicle ~4% ethanol in saline solution). *In vivo* haemodynamic measurements were taken for right ventricular systolic pressures (RVSP) by transdiaphragmatic puncture under 1.5% isofluorane anaesthesia. Pulmonary vascular remodelling (PVR) was determined in lung sections by blind-assessment of the number of remodelled arteries in the distal vasculature. Proliferation was assessed in female human pulmonary arterial smooth muscle cells (hPASMCs) in response to 72 hour incubations with the predominant CYP1B1 and COMT metabolites, 4-OHE1 and 2-MeOHE2, respectively, by thymidine incorporation and ATP assays (CellTiter® Glo, Promega). Statistical analysis was performed with a one-way ANOVA followed by a Bonferroni's post-hoc test. Data is expressed as mean ± SEM.

Results Aromatase and CYP1B1 protein expression were increased in female SERT+ lungs compared to their wildtype controls (5.62 ± 0.77 and 1.31 ± 0.11 fold higher, $P<0.01$, $P<0.05$ respectively). Whole lung E2 levels were unchanged in SERT+ mice (0.49 pg/μg ± 0.02 c.f. 0.46 pg/μg ± 0.02, respectively, n=4-5). Inhibition of CYP1B1 with TMS attenuated the increased RVSP in female SERT+ mice (24.42mmHg ± 0.68 c.f. 21.43mmHg ± 0.88 n=9-10 $P<0.01$) and PVR (16.75% ± 1.72 c.f. 8.17% ± 1.58, n=5-6 $P<0.05$ SERT+ c.f. SERT+ TMS). 4-OHE1 and 2-MeOHE2 inhibited thymidine incorporation by 83.00% ± 1.51 and 68.36% ± 1.93, respectively (n=7-8, $P<0.001$), but only 2-MeOHE2 reduced cell viability by 15.38% ± 4.38 ($P<0.01$, n=7-8). Activity of COMT may rapidly convert 4-OHE1 to its methylated metabolite. Thus we investigated the effect of 4-OHE1 in the presence of a COMT inhibitor, OR-

486 (10μM). In the presence of OR-486, 4-OHE1 caused proliferation of hPASMCs ($n=7\text{-}8$, $P<0.05$).

Conclusion Here, we provide evidence for a dysregulated E2 axis in female SERT+ mice that can be beneficially rescued by CYP1B1 inhibition. This further supports the application of E2-related therapies in the treatment of this highly gender-selective disease.

1. White, K et al, Cardiovascular Research, 90:373, 2011

2. White, K et al, Circulation, 126:1087, 2012