IRAP Inhibition - A disease-modifying treatment for Pulmonary Arterial Hypertension

Supitchaya Watakul[1], Peng-Cheng Wang[1], Jana Goldenberg[1], Robert E Widdop[1], Barbara K Kemp-Harper[1] and Tracey A Gaspari[1]. Dept of Pharmacol, Monash University[1], Clayton, VIC, Australia.

Background

Pulmonary Arterial Hypertension (PAH) is an incurable and fatal disease with well-established sexual dimorphism, being four times more prevalent in females, yet males have more severe disease and higher mortality (Farber et al., 2015). Current therapies are mostly vasodilators, some of which display sex-specific efficacy, such as sildenafil/tadalafil being more effective in males (Mathai et al., 2015). These therapies provide symptomatic relief but have limited impact on the underlying inflammation and remodelling, highlighting the need for treatments effective across both sexes that can halt or reverse pulmonary vascular and right ventricular (RV) remodelling. Insulin-regulated aminopeptidase (IRAP) has emerged as a promising target, with IRAP inhibition demonstrating vasoprotective, anti-inflammatory and anti-fibrotic effects in various cardiovascular disease models (Chai et al., 2018), yet its role in the pulmonary circulation remains unexplored and is the focus of the current study.

Hypothesis/Aims

We hypothesised that IRAP inhibition using the well-characterised IRAP inhibitor, HFI-419, would halt or reverse inflammation and pathological remodelling in the gold standard sugen-hypoxia murine model of PAH. This study aimed to determine whether HFI-419 reduces inflammation, pulmonary vascular and RV remodelling more effectively than the current standard-of-care, sildenafil, in both male and female mice.

Methodology

PAH was induced in male and female C57BL/6J mice using the sugen-hypoxia model (SuHx; 42 days of 10% O_2 exposure; 20 mg/kg sugen given s.c. at days 0, 7 and 14). To determine changes in IRAP expression over PAH development, lung IRAP immunofluorescent staining was performed at different timepoints (14, 21, 35 and 42 days; n=6-9/timepoint) during PAH development and compared to normoxic (NmOx) controls. Subsequently, an intervention study was performed where SuHx mice were randomised to receive treatment commencing at day 21 (n=6-10/group): HFI-419 (0.72 mg/kg/day; s.c. minipump) or sildenafil (30 mg/kg/day; oral). Endpoint measures included right ventricular systolic pressure (RVSP), right ventricular hypertrophy (RVH), histological analysis of pulmonary vascular remodelling, macrophage infiltration and IRAP expression, qPCR analysis of CCL2 and IL6 and plasma IL6 quantification.

Results

Baseline lung IRAP expression was ~6-fold higher in females than in males. During PAH development, IRAP expression increased almost 10-fold in SuHx male mice at day 42 compared to NmOx ($P<0.0001$), but did not increase in females, resulting in similar IRAP expression between sexes at day 42.

In the intervention study, the SuHx model significantly increased RVSP (Male: NmOx 28.1±1.0 vs SuHx 44.3±1.1 mmHg and Female: NmOx 23.5±1.0 vs SuHx 43.6±1.3 mmHg; $P<0.0001$), RVH and pulmonary vessel wall thickness (Male: 82% increase from NmOx and Female: 118% increase from NmOx; $P<0.0001$) in both sexes. In male SuHx mice, both sildenafil and HFI-419 reduced RVSP compared to

SuHx (40.0±1.0 and 38.4±0.7 vs 44.3±1.1 mmHg; P<0.05). By contrast, neither treatment reduced RVSP in females, and RVH was unaltered in either sex.

Notably, IRAP inhibition significantly reduced pulmonary vessel wall thickness by ~20% in both sexes from SuHx (P<0.01), an effect not observed with sildenafil. Lung macrophage number in NmOx was ~65% higher in females compared to males but was similar between sexes at day 42. Despite these sex differences, HFI-419 reduced macrophage infiltration in male SuHx lung to NmOx levels (P<0.01) with a similar effect in CCL2 gene expression (P<0.001). HFI-419 also reduced lung IL6 gene expression (P<0.05) and trended to reduce plasma IL6 levels in male SuHx mice.

Conclusions

IRAP inhibition shows promise in reducing pulmonary vascular remodelling in PAH across both sexes, currently unaddressed by standard-of-care therapies. In male mice, HFI-419 also reduced inflammatory gene expression and macrophage infiltration, with equivalent analyses in females underway. Consistent with clinical observations, sildenafil lowered RVSP in male but not female mice, and a similar sex-specific pattern was observed with HFI-419. Future studies will explore the efficacy of IRAP inhibitors in combination with standard-of-care therapies and define the mechanisms driving these anti-inflammatory and anti-remodelling effects. Collectively, this study provides the first evidence supporting IRAP inhibition as a potential disease-modifying treatment for PAH.

Statement of the scientific innovation and significance of the study

This study is the first to investigate IRAP as a therapeutic target in PAH, shifting the focus from symptomatic relief to reversing pulmonary vascular remodelling. We show that IRAP is a viable target, offering a disease-modifying approach beyond pressure reduction. Given the well-established sex differences in PAH prevalence, severity and treatment response and the predominance of male-only preclinical studies, we evaluated IRAP inhibitors in both sexes to address this critical gap.

Statement on the nominating student's specific role in the study

The nominating student has performed >90% of all experimental work and data analysis. This study forms part of her PhD project.

Declaration

We declare that this document was prepared primarily by the nominating student and has undergone minimal corrections by the supervisor or any other author on the accompanying abstract.

Student's name: Supitchaya Watakul Student's signature:  Date: 08/05/2026

Supervisor's name: Tracey Gaspari Supervisor's signature:  Date: 08/05/2026

References

Farber, H. W., Miller, D. P., Poms, A. D., Badesch, D. B., Frost, A. E., Rouzic, E. M.-L., … Benza, R. L. (2015). Five-Year Outcomes of Patients Enrolled in the REVEAL Registry. CHEST, 148(4), 1043-1054. doi: https://doi.org/10.1378/chest.15-0300

Mathai, S. C., Hassoun, P. M., Puhan, M. A., Zhou, Y., & Wise, R. A. (2015). Sex Differences in Response to Tadalafil in Pulmonary Arterial Hypertension. CHEST, 147(1), 188-197. doi:10.1378/chest.14-0263

Chai, S.Y., Widdop, R.E., Gaspari, T.A. & Lee, H.W. Fibrotic treatments. (2018).