Impact of treprostinil on dynamin-related protein 1 (DRP1) and mitochondrial fragmentation in pulmonary arterial hypertension (PAH).

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Background: Prostacyclin analogues like treprostinil are used clinically to treat PAH, a, progressive, life-threatening disease of the pulmonary vasculature. Central to the pathogenesis of PAH is proliferation of pulmonary arterial smooth muscle cells (PASMCs) within the medial layers of small pulmonary arteries. Increased PASMC proliferation is associated with mitochondrial fragmentation due to heightened activity of DRP1.

Aim: To elucidate whether treprostinil acting on IP and/or EP2 prostanoid receptors affects DRP1 and mitochondrial dynamics in PASMCs from patients with PAH.

Methods: PASMCs were cultured with treprostinil (100nM) for 3 hours alone or with either the IP (RO1138452; 1µM) or EP2 (PF04418948; 1µM) receptor antagonist, the PKA inhibitor H-89 (10µM) or a combination. DRP1 phosphorylation on serine 637 (pDRP1S637) was determined by western blotting. Live-cell imaging with MitoTracker Red CM-H2Xros assessed mitochondrial morphology**.**

Results: Low levels of pDRP1S637 were observed in untreated PASMCs, while treprostinil markedly increased the levels of pDRP1S637 ($n=3$, $P<0.05$) and promoted mitochondrial fusion and elongation. Although, individually, RO1138452 and PF04418948 had no effect on treprostinil-induced pDRP1S637, together they significantly blocked treprostinil-induced phosphorylation of DRP1 ($n=3$; $P<0.01$). Treprostinil-induced DRP1 phosphorylation was also abolished by H-89 ($n=3$; $P<0.0001$).

Conclusions: These results suggest that treprostinil can signal via either the IP or EP2 receptors to activate PKA and phosphorylate DRP1 on the inhibitory residue serine 637, thus promoting mitochondrial fusion and elongation**.**