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Effects of connexin mimetic peptide Gap27 on hypoxic-induced proliferation and migration of rat pulmonary artery fibroblasts

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Introduction: Pulmonary arterial hypertension (PAH) is a disease associated with remodelling of the pulmonary arteries and increased pulmonary vascular resistance resulting in right ventricular dysfunction[1]. Pulmonary artery fibroblast (PAF) proliferation and migration contributes to vascular remodelling. Mitogen Activated Protein Kinase (MAPK) signalling pathways are known to contribute to PAF proliferation and migration[2]. Dysfunctional connexin-mediated signalling is thought to play a role in the pathophysiology of PAH[3]. The aim of this study was to investigate the effects of Gap27, a connexin mimetic peptide and specific inhibitor of connexin 43 and 37 on rat PAF (rPAF) proliferation, migration and MAPK protein expression.

Methods: rPAFs were exposed to hypoxic ($5\%O_2$) or normoxic conditions for 24 hours in the presence or absence of Gap27 (300µM). Cells were counted using the Countess II (Life Technologies) to assess proliferation. Scratch assays were conducted in order to assess migration. Protein was harvested and expression of phosphorylated p38 (pp38) and phosphorylated ERK (pERK) were assessed using Western blot. Data was analysed using two way ANOVA followed by Bonferroni's post-hoc test or an unpaired t-test.

Results: Hypoxia caused an increased proliferation of rPAFs which was inhibited by Gap27 (**Figure1**). Hypoxia also significantly increased cell migration, an effect which was attenuated by Gap27 (**Figure2**). Gap27 inhibited hypoxic-induced increases in protein expression of pp38 (426.95% ± 62.24% vs 136.82% ± 32.4% n=3, $p<0.001$) and pERK 1/2 (259.72% ± 5.28% vs 44.79% ± 24.99% n=3, $p<0.001$) in rPAFs.

Figure 1. Effects of Gap27 on hypoxic-induced proliferation of rPAFs. Data expressed as mean ± SEM. **p< 0.01 ***p<0.001.

Figure 2. Effects of Gap27 on hypoxic-induced migration of rPAFs. Data expressed as mean ± SEM. **p< 0.01 ***p<0.001.

Conclusions: Our results show connexins play a role in hypoxic-induced proliferation and migration of rPAFs via activation of the MAPK pathway.

References:

1. Simonneau, G et al. (2004). *J Am Coll Cardiol*, **43**, 5S-12S.
2. Dempsie, Y et al. (2015). *Biochem Soc T*, ***43*(3)**, 524-529.
3. Welsh, D. J et al. (2001). *Am J Respir Crit Care Med*, ***164***, 282-289.

Figure1 Effects of Gap 27 on hypoxia-induced proliferation of rPAFs (n=12)

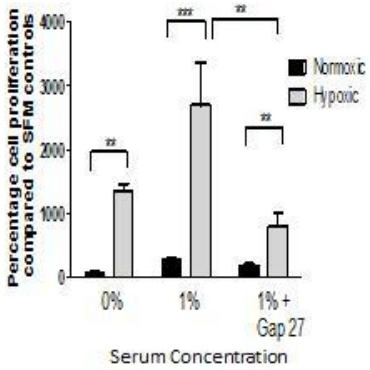

Figure2 Effects of Gap27 on hypoxic-induced migration of rPAFs (n=4)