Effects of ASK-1 inhibition on pulmonary artery fibroblast proliferation and migration from SUGEN/hypoxic rats

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Introduction: Pulmonary artery fibroblast (PAF) proliferation contributes to the vascular remodelling seen in pulmonary arterial hypertension (PAH)[1].Oxidative stresses seen in PAH activates ASK-1 which undergoes auto-phosphorylation and activation of downstream p38-MAPK[2]. The aim of this study was to investigate the effects of ASK-1 inhibition on rat PAF (rPAF) proliferation, migration and p38 protein expression.

Methods: rPAF cells (P3-P5) were isolated from either normoxic, SUGEN/hypoxic or SUGEN/hypoxic male sprague dawley rats fed with either control chow or chow containing an ASK-1 inhibitor (GS-444217, 3-10µM). Rats were dosed with Sugen (20mg/kg) before 2 weeks of hypobaric hypoxia then 3 weeks normoxia. Cells were counted using the Countess II (Life Technologies) to assess proliferation. Scratch assays were conducted in order to assess migration. Expression of phosphorylated p38 (pp38) was assessed using Western blot. Data was analysed using two way ANOVA followed by Bonferroni's post-hoc test or an unpaired t-test.

Results: Fibroblasts from SUGEN/hypoxic rats displayed increased proliferation to 1% and 5% serum which weren't seen in the fibroblasts from rats treated with GS-444217 (**Figure1**). Fibroblasts from SUGEN/hypoxic rats also displayed significantly increased cell migration, an effect which wasn't seen in the animals treated with GS-444217 (**Figure1**). ASK-1 inhibition prevented SUGEN/hypoxic-induced increases in protein levels of pp38 (446.88% ± 55.40% vs 174.30% ± 79.90% n=3, p<0.001) in rPAFs **Figure 1. Effects of ASK-1 inhibition on SUGEN/hypoxic -induced proliferation and Migration of rPAFs**. Data expressed as mean ± SEM. *P< 0.05 ***p<0.001.

Conclusions: Our results show that rPAFs harvested from SUGEN/hypoxic rats display an enhanced proliferative phenotype over those isolated from control rats. Furthermore, cells isolated from SUGEN/hypoxic rats treated with an ASK-1 inhibitor display reduced proliferative and migratory phenotypes via the p38 MAP kinase signalling pathway.

References:

1. Welsh, D. J et al. (2001). *Am J Respir Crit Care Med*, ***164***, 282-289.
2. Wilson, K et al (2015). Heart, 101:A14-A15.

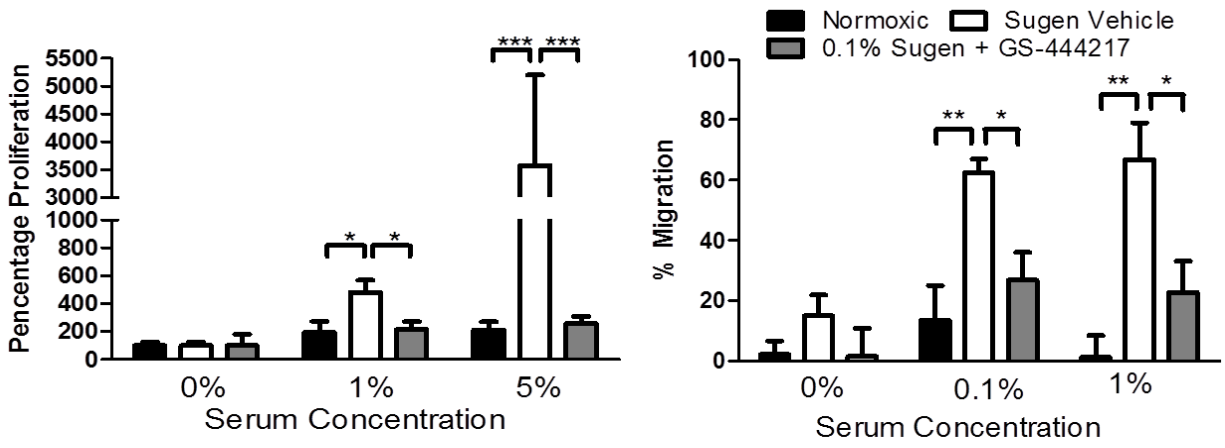

Figure 1. Effects of ASK-1 inhibition on SUGEN/hypoxic -induced proliferation and Migration of rPAFs. Data expressed as mean ± SEM. *P< 0.05 ***p<0.001.