

**Introduction** VEGF has been implicated in the development of IPF. Alternative splicing of the VEGF-A gene generates numerous isoforms. The differential effects of these isoforms, in particular the VEGF-A<sub>xxx</sub>b family, thought to have several opposing functions to the conventional family of isoforms (VEGF-A<sub>xxx</sub>a), have not been considered.

#### Hypothesis

- The balance of VEGF-A<sub>xxx</sub>a:VEGF-A<sub>xxx</sub>b isoform expression is important in the pathogenesis of IPF.
- VEGF-A<sub>xxx</sub>b isoforms may be protective against the formation of pulmonary fibrosis (PF).

**Methods** Normal and IPF lung lysates (n = 5) were analysed by western blotting (WB), and ELISA using an antibodies specific for PanVEGF-A and VEGF-A<sub>xxx</sub>b isoforms.

The Bleomycin (BLM)-induced model of PF was used in conjunction with two transgenic (TG) mouse models, developed to explore the role of ATII-derived VEGF in the development of PF: 1) a conditionally inducible, ATII-specific, VEGF knock-out mouse (STCLL mice) and 2) a TG mouse over-expressing VEGF-A<sub>xxx</sub>b in ATII cells (MMTV-VEGF<sub>165</sub>b).

To explore the therapeutic potential of VEGF-A<sub>xxx</sub>b in PF, wild-type mice were administered intraperitoneal (IP) injections of VEGF-A<sub>165</sub>b, commencing 10 days after BLM challenge.

In all experiments fibrosis was assessed histologically using Masson's Trichrome, with blinded scoring of tissue sections.

**Results** By WB (n = 3) and ELISA (n = 5) there was no significant difference in PanVEGF-A expression between normal and IPF lung homogenates (*t*-test, *p* > 0.05). In contrast, VEGF-A<sub>xxx</sub>b expression was significantly increased in these same IPF samples compared to control, by ELISA (*t*-test, \*\*\*\**p* < 0.0001) and WB (Densitometry: *t*-test, \**p* < 0.05).

Specific deletion of VEGF-A from ATII cells of mice ameliorated the development of BLM-induced pulmonary fibrosis (n = 5, Lung fibrosis score: ANOVA with Holm's Sidak \*\**p* < 0.01). Over-expression of VEGF-A<sub>xxx</sub>b in ATII cells also ameliorated the development of pulmonary fibrosis (n = 6, Lung fibrosis score: ANOVA with Holm's Sidak \*\*\**p* < 0.001). Furthermore, delivery of VEGF-A<sub>165</sub>b, specifically during the fibrotic phase of the BLM model, also attenuated lung fibrosis development (n = 6, Lung fibrosis score: ANOVA with Holm's Sidak \**p* < 0.05). **Conclusion** Changes in the bioavailability of ATII cell-derived VEGF-A, namely the ratio of VEGF-A<sub>xxx</sub>a:VEGF-A<sub>xxx</sub>b, appear critical to the development of pulmonary fibrosis. This data suggests that more a targeted approach to anti-VEGF-A therapy in IPF should be explored.

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#### THE ROLE OF SRC KINASE IN INSPIRATORY RESISTIVE BREATHING-INDUCED PULMONARY INFLAMMATION

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**Introduction and objectives** Inspiratory resistive breathing (IRB), a hallmark of obstructive pulmonary diseases, is characterised by large negative intrathoracic pressures. IRB is shown to induce pulmonary inflammation in previously healthy rats. Src is a multifunctional kinase that is activated by phosphorylation upon mechanical stress and plays a significant role in inflammatory processes. The aim of our study was to investigate the role of Src in IRB-induced pulmonary inflammation.

**Methods** Anaesthetised, tracheostomised rats were breathing spontaneously through a 2-way non rebreathing valve. The inspiratory port was connected to a resistance, setting peak tidal tracheal pressure at 50% of maximum (IRB). Quietly breathing animals served as controls. After 6 h of IRB, the mechanics of the respiratory system were assessed with the forced oscillation technique. Bronchoalveolar lavage (BAL) was performed to measure total and differential cell count and total protein levels. Phosphorylation of Src and ERK was detected in lung tissue samples by Western blot analysis at 30 min, 3 and 6 h of IRB. The Src inhibitor PP2 was administered intraperitoneally (1 mg/kg), 30 min prior to IRB, in a subgroup of animals.

**Results** After 6 h of IRB, increased tissue elasticity was measured, compared to control. Increased BAL cellularity was also found (2-fold increase to control), due to raised numbers of both macrophages and neutrophils. Total protein levels were elevated in BAL fluid. Src activation was detected at 30 min of IRB (3-fold increase to control), while ERK was phosphorylated at 3 and 6 h. Inhibition of Src kinase attenuated the increase in tissue elasticity after 6 h of IRB. Following inhibition of Src kinase, the total cell number after 6 h of IRB was not increased compared to control. Neither macrophage nor neutrophil count was elevated after 6 h of IRB, following Src inhibition. Total protein levels were not altered by Src inhibition. Src inhibition attenuated the activation of ERK only at 3 h of IRB.

**Conclusion** Src kinase activation partly mediates IRB-induced pulmonary inflammation.

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#### CAFFEINE INHIBITS TGFβ ACTIVATION BY EPITHELIAL CELLS, INTERRUPTS FIBROBLAST RESPONSES TO TGFβ, AND REDUCES PULMONARY FIBROSIS IN EX VIVO PRECISION-CUT LUNG SLICES

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Caffeine (1, 3, 7-tri-methylxanthine) is a common food additive found naturally in many products. It is a non-selective competitive antagonist of G-protein coupled adenosine receptors and can inhibit phosphodiesterases. Caffeine has anti-fibrotic effects in the liver and increased caffeine consumption has been associated with reduced liver fibrosis in patients with chronic hepatitis C infection. The effect of caffeine on pulmonary fibrosis has not been investigated, however, it has been shown to inhibit TGFβ-induced Smad signalling in epithelial cells. This study aimed to investigate the anti-fibrotic effects of caffeine in the lung using lung epithelial cells, fibroblasts and an *ex vivo* precision-cut lung slice (PCLS) model of fibrosis.

Immortalised human bronchial epithelial cells (iHBEs) and primary human lung fibroblasts were used. TGFβ activation was assessed using an *in vitro* TGFβ reporter cell assay and assessment of phosphorylated Smad2. Expression of pro-fibrotic genes was assessed by quantitative polymerase chain reaction. Proliferation of fibroblasts was assessed by brdU incorporation assay. Finally, the effect of caffeine on established lung fibrosis was investigated *ex vivo* using PCLS. Mice were instilled with saline or 60 IU bleomycin and PCLS obtained after 28 days. PCLS were treated with increasing concentrations of caffeine for five days prior to measurement of collagen by high-performance liquid chromatography. Viability of the PCLS following caffeine treatment was assessed by MTT assay.