Endothelin and scleroderma lung disease

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Scleroderma-associated interstitial lung disease (SSc-ILD) occurs frequently and for many patients SSc-ILD is a significant complication of their disease. SSc-ILD is now one of the leading causes of death among patients with SSc. SSc-ILD, classified most often as a non-specific interstitial pneumonia, may culminate in interstitial pulmonary fibrosis and end-stage lung disease. Fibrosis in the lung is the net result of fibroblast proliferation and deposition of excessive amounts of extracellular matrix proteins. Among the many cytokines and growth factors involved in the pathogenesis of SSc-ILD, ET-1 may be a central mediator. In vitro and in vivo studies of animals and SSc patients support the notion that ET-1 can enhance the proliferation of pulmonary mesenchymal cells and may also enhance the fibrogenic effects of TGF-β. Two well-designed randomized controlled trials of the dual ET receptor blocker bosentan were negative in their primary (and for SSc also secondary) endpoints, although there might be explanations for this apparent lack of efficacy.

**Key words:** Endothelin, Scleroderma, Interstitial lung disease, Pulmonary fibrosis.

**Introduction**

Best known for its role as a potent vasoconstrictor, ET-1 has also been shown to be a mediator of fibrosis. There is evidence that ET-1 plays a role in the pathogenesis of fibrosis in a number of organs including the skin, heart, kidney, liver and lung. Since interstitial lung disease (ILD) is a frequent and often dominant complication of SSc (scleroderma), it is appropriate to review the clinical and in vitro data pertaining to ET-1 and its role in the pathogenesis of scleroderma lung disease (SSc-ILD). Elsewhere, ET-1’s role in the pathogenesis of SSc-pulmonary arterial hypertension (PAH) is discussed.

**ET as a mediator of lung fibrosis**

Fibrosis is the net result of an increase in fibroblast proliferation and the associated accumulation of extracellular matrix (ECM) proteins. Data suggest that ET-1 can induce fibrosis directly by binding to ETA and to ETβ receptors on fibroblasts, or indirectly by inducing fibrogenic cytokines such as TGF-β. Various animal models have been employed to demonstrate a role for ET-1 in lung fibrosis. For example, transgenic mice overexpressing ET-1 spontaneously develop lung fibrosis in conjunction with the accumulation of perivascular inflammatory cells, mostly CD4-positive cells [1]. Also, ET-1 levels are elevated in bleomycin-induced pulmonary fibrosis; the increase in ET-1 occurs prior to an increase in collagen content and is localized within developing fibrotic lesions [2]. Some groups have reported that treatment with bosentan, a dual ET receptor antagonist, attenuates bleomycin-induced pulmonary fibrosis in rats where ET-1 and both the ETA and ETβ receptors are up-regulated on fibroblasts and infiltrating monocytes [3]. Others have not shown an effect of either bosentan or the selective ETA receptor antagonist BQ-485 on collagen accumulation in this model [4]. Additionally, in a rodent model of myocardial infarction, lung fibrosis was not prevented by the selective ETα receptor antagonist LU135252, despite a significant drug effect on reducing right ventricular systolic pressure [5]. Hence, the ETα receptor may not be involved in lung fibrosis in these animal models, or other factors such as TGF-β may play a more central role.

Epithelial cell injury and the process of epithelial–mesenchymal transition (EMT) are important events in the pathogenesis of pulmonary fibrosis, and recent evidence links ET-1 with this process of EMT. Jain et al. [6] demonstrated that primary alveolar epithelial cells produce physiologically relevant amounts of ET-1 in vitro and express ETA and ETβ receptors, suggesting an autocrine or paracrine function for alveolar ET-1. Furthermore, it was shown that ET-1 induces EMT via ETA activation. ET-1 increased TGF-β, and ET-1-induced EMT was attenuated by a TGF-β1-neutralizing antibody [6]. In addition to increasing the level of TGF-β, acting through its G protein-coupled receptor ET-1 activates TGF-β signalling and affects loss of an epithelial marker (pro-surfactant protein B) and gain of a mesenchymal marker (alpha-smooth muscle actin, α-SMA). When added to normal lung fibroblasts in vitro, ET-1 induces expression of α-SMA and other proteins that contribute to the contractile phenotype of myofibroblasts present in SSc-ILD and other fibrosing conditions.

Not only does ET-1 increase TGF-β and activate TGF-β signalling, but it also appears that TGF-β induces ET-1 in human lung fibroblasts. This induction of ET-1 occurs through a Smad-independent, activin receptor-like kinase 5 (ALK-5)/JNK-dependent mechanism and an activator protein 1 (AP-1) site in the ET-1 promoter [7]. Recently, it has been suggested that ET-1 contributes to the ability of TGF-β to promote a profibrotic phenotype in human lung fibroblasts [8]. Dual ETA and ETβ receptor blockade with bosentan attenuated the induction of type I collagen, fibronectin, TIMP-3 and CCN-2 by TGF-β in human lung fibroblasts [8].

**ET and SSc-ILD**

ET-1 plasma concentrations are elevated in SSc and are highest in patients with the diffuse cutaneous form of the disease. A study of a small sample of SSc patients confirmed the elevation of plasma ET-1 in SSc, yet failed to demonstrate an association with SSc-ILD [9]. However, ET-1 concentrations are significantly elevated in plasma of patients with idiopathic pulmonary fibrosis (IPF), as...
well as in the bronchoalveolar lavage fluid and breath condensate from SSc patients when compared with healthy subjects [10].

Multiple sources probably account for the observed increase in lung ET-1 concentrations in SSc-ILD patients, including epithelial cells, alveolar macrophages, endothelial cells and mesenchymal cells. As noted earlier, alveolar epithelial cells produce ET-1 in vitro [6], and alveolar epithelial injury is a common feature of many types of ILD including SSc-ILD. LPS-stimulated alveolar macrophages from SSc patients secrete higher amounts of ET-1 than control subjects [11]. Using immunohistochemical staining, Abraham et al. [12] reported that SSc patients with ILD demonstrate expression of ET-1 on airway bronchiolar and alveolar epithelium and interstitium, interstitial vessels (including endothelial cells) and alveolar macrophages. The same authors reported an overall increase in ET receptor expression in SSc-ILD lung tissue, with a differential expression showing a significantly reduced level of the ETa receptor with a slightly increased level of the ETb receptor [12].

**Implications for treatment of SSc-ILD**

Animal models of lung fibrosis together with the clinical and histological observations cited above support a major role for ET-1 in the pathogenesis of SSc-ILD. Blockade of ET-1 and its fibrogenic effects would thus seem to be a logical approach to the treatment of SSc-ILD. Any salient effect of ET receptor blockade on lung fibrosis must, however, be balanced by a potentially deleterious effect on gas exchange, whereby a systemically administered vasodilator interferes with ventilation/perfusion matching. In a 12-week study of 12 IPF patients, bosentan induced clinically relevant gas exchange abnormalities in only one subject [13], and thus ET receptor blockade is likely to be tolerated by most patients with SSc-ILD. A placebo-controlled trial of bosentan in SSc-ILD patients, the BUILD-2 (Bosentan in Interstitial Lung Disease in Systemic Sclerosis-2) study, was halted after no effect was observed on the primary endpoint (6-min walk distance, 6MWD), nor on any of the secondary endpoints including FVC, DLCO and time to desaturation. Likely explanations for the failure of this clinical trial include the fact that 6MWD is not a sensitive endpoint in SSc-ILD patients, as well as the possibility that the study population had fairly stable ILD that would not likely be responsive to therapy. Recently, the results of BUILD-1, a placebo-controlled trial of bosentan in patients with IPF, have been published [14]. Similar to the results in SSc-ILD, bosentan showed no superiority over placebo in 6MWD up to 12 months. A trend in favour of bosentan was, however, observed for the secondary endpoint of time to death or disease progression, and the trend was more pronounced in a patient subgroup diagnosed using surgical lung biopsy. Changes from baseline up to month 12 in assessments of dyspnoea and quality of life favoured treatment with bosentan. Additional studies of ET receptor blockade in SSc-ILD patients would appear to be warranted, perhaps with more stringent inclusion criteria to select patients whose lung disease is progressing, and perhaps using primary and secondary endpoints which might be more responsive to change. The identification of biomarkers of ongoing lung fibrosis would enhance our ability to select patients for clinical trials, and the identification of responsive measures of lung response would facilitate the design of clinical trials of ET blockers and other agents for the treatment of SSc-ILD.

**Rheumatology key messages**

- ET-1 is an important mediator of fibrosis by inducing the proliferation of mesenchymal cells and by affecting the actions of TGF-β.
- ET-1 levels are elevated in animal models of lung fibrosis as well as in patients with SSc-ILD.
- ET receptor blockade has been shown to ameliorate some but not all animal models of lung fibrosis.
- A clinical role for ET receptor blockade has not yet been demonstrated for SSc-ILD, although recently reported data suggest potential efficacy in some patients with IPF.
- Based on animal studies and clinical observations, blocking the actions of ET-1 has potential therapeutic implications for SSc-ILD; however, a randomized controlled trial of bosentan failed to meet the primary and secondary endpoints.

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**References**