Commentary on a recent article—“A prostacyclin analogue, Iloprost, protects from bleomycin-induced fibrosis in mice” Zhu Y et al. *Respir Res.* 2010 Mar 20;11(1):34

Richard Stratton · Florence Newton

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Abstract Data from our laboratory show that in vitro fibroblasts are exquisitely responsive to prostacyclin and the prostacyclin derivative Iloprost, which block their activation by TGF β . A recent article by Zhu Y et al confirm these effects in vivo showing that Iloprost, given as a single intraperitoneal injection, blocks lung fibrosis in the bleomycin model of lung injury and fibrosis. These results are important because at present no effective clinical treatments are available to treat idiopathic lung fibrosis, which progresses and leads to respiratory failure. Limiting factors for the clinical use of prostacyclin derivatives as anti-fibrotics are failure to achieve therapeutic levels in the involved fibrotic tissues, and dose limiting side effects due to vasodilatation and binding to the IP receptor on vascular cells. Possible approaches include fibroblast directed gene therapies or amelioration of the vascular side effects.

Keywords Fibrosis · Iloprost · Prostacyclin

We have an interest in the potential anti-fibrotic effects of Iloprost because we use it in patients with systemic sclerosis (SSc), originally as a therapy for vascular disease (Varga and Abraham 2007). In SSc vascular manifestations are among the earliest symptoms, and Iloprost and other IP receptor, analogues have been shown to decrease the severity and frequency of attacks of Raynaud's (reviewed in (Nuttall et al. 2010)). Because of this intravenous Iloprost given daily for 5 days has become standard

treatment for SSc patients and does help protect against critical digital ischaemia in the disease (Scorza et al. 2001).

A number of our patients noticed that their skin tightness due to skin fibrosis improves symptomatically following 5 days of Iloprost. We became interested in the idea that Iloprost might have some clinically useful anti-fibrotic effect, and so we started to test whether Iloprost could inhibit fibroblastic responses in vitro. Our system uses dermal fibroblast induction by TGF β in tissue culture as a model of pro-fibrotic responses. We found that Iloprost, at levels as low as 100 pg/ml, was able to block the induction of type I collagen and CTGF in fibroblasts exposed to TGF β (Stratton et al. 2001). In fact the plasma levels of Iloprost achieved during infusion of the drug in standard doses approaches this level.

The pharmacokinetics of Iloprost have been studied extensively in humans. In one study, plasma levels were measured in healthy male volunteers given 1 and 3 ng/kg/min intravenous for 45 min, and 1 microgram/kg orally once per day (Krause and Krais 1986). Following intravenous infusion, the mean steady-state plasma levels of Iloprost were 46 pg/ml and 135 pg/ml for the two respective intravenous dosing schedules. In our experience many of the SSc patients only tolerate low level use of Iloprost and develop headaches, flushing, and diarrhoea at doses above 1 ng/kg/min. After oral administration, absorption of the drug is rapid, with a maximum plasma level of 251 $\pm$ 32 pg/ml being achieved after 10 $\pm$ 6 min, and rapidly declining thereafter (Hildebrand 1997).

Pharmacokinetics of Iloprost have also been studied using radiolabelled Iloprost, given orally and by intravenous infusion to elderly volunteers (Krause and Krais 1987). Plasma levels were measured after intravenous infusion of 2 ng/kg/minute for 4 h, and oral administration of 0.1 and 0.48 μ g/kg in healthy elderly subjects. After intravenous

R. Stratton (✉) · F. Newton
Centre for Rheumatology and Connective Tissue Diseases
Research, Royal Free Hospital, UCL,
Rowland Hill Street,
London NW3 2PF, UK
e-mail: r.stratton@medsch.ucl.ac.uk

infusion at 2 ng per kg per minute a steady-state of unchanged Iloprost was reached rapidly with a peak of 81 pg/ml. Plasma levels declined biphasically after termination of the infusion, with half-lives of 6 min and 31 min. Our conclusion was that in standard usage the plasma levels of Iloprost achieved approach those found by us to suppress fibroblast activation in vitro, and we went on to test whether the levels of dermal interstitial CTGF were suppressed during routine use of the drug in SSc patients. We found moderate suppression of CTGF during Iloprost infusing in some, but not all patients studied (Stratton et al. 2001).

We went on to show the mechanism by which Iloprost blocks CTGF induction and that this effect is due to elevation of cAMP in fibroblasts and due to the PKA-dependent suppression of Ras/MEK/ERK signalling (Stratton et al. 2002). We also made some studies in vivo using a subcutaneous chamber model of skin granulation tissue in the rat. Injection of the cylinders with Iloprost was found to block CTGF and type I collagen induction by TGF β in vivo.

In their recent article, Zhu and colleagues have assessed whether Iloprost given as a single intraperitoneal injection blocks the development of lung fibrosis following intratracheal bleomycin (Zhu et al. 2010). These studies reveal that Iloprost, when administered before the bleomycin, blocks the gross fibrotic changes seen after 14 days, and has a number of other interesting effects including antagonism of inflammatory cell recruitment and suppression of pro-inflammatory mediators such as IL6 and TNF α . It remains to be shown whether these beneficial effects are downstream of the inhibition of fibroblast activation, or whether they represent additional effects of the drug outside direct inhibition of fibroblast responses to TGF β . One possibility is that by blocking differentiation to myofibroblasts capable of destructive fibrosis, Iloprost is inhibiting fibroblast-dependent release of chemokines and cytokines, leading to downstream loss of inflammatory cell recruitment and activation.

There certainly is a clinical need for effective anti-fibrotic strategies and the question remains as to whether Iloprost or other IP receptor agonists have therapeutic potential in patients suffering fibrotic conditions. In some of our SSc patients Iloprost is used by continuous IV infusion over months or years as a therapy for pulmonary vascular disease (vascular intimal hyperplasia causing pulmonary hypertension), and in this context we have seen

patients progress despite the treatment in terms of lung and skin fibrosis. Based on the above studies we concluded that with the doses tolerated during intravenous infusion of the drug, the plasma levels are at or slightly below the threshold required for a clinically useful anti-fibrotic effect, and that the levels achieved within the relatively avascular fibrotic interstitium may be below that required to abolish CTGF induction for example. As the therapeutic use is limited by vasodilator related side effects such as hypotension, flushing and headaches, it may be possible to target the therapy to fibroblasts or to modify the vascular responses to allow for higher intravenous dosing. For example, using gene therapy based on the type I collagen gene promoter linked to prostacyclin synthase, therapeutic levels of prostacyclin could be achieved within areas of fibroblast activation.

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