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LU135252, an endothelin$_A$ receptor antagonist did not prevent pulmonary vascular remodelling or lung fibrosis in a rat model of myocardial infarction

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1 The early intervention with endothelin$_A$ (ET_A) receptor antagonists following coronary artery ligation has been shown to reduce the development of pulmonary hypertension, despite a lack of improvement in left ventricular function.

2 The present study examined the contribution of pulmonary vascular remodelling and the progression of lung fibrosis in the development of pulmonary hypertension and the subsequent role of endothelin-1 in these processes in a rat model of myocardial infarction (MI).

3 The administration of 60 mg kg^{-1} per day of the specific ET_A receptor antagonist LU135253 ((+)-(S)-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid) 24 h following coronary artery ligation, failed to improve left ventricular contractile indices, but reduced the extent of pulmonary hypertension, as reflected by the significant decrease in right ventricular systolic pressure.

4 The medial wall thickness of small pulmonary arteries (50–200 μm) was significantly increased 4 weeks following MI, albeit LU135253 treatment did not ameliorate this pattern of vascular remodelling.

5 The steady-state mRNA levels of collagen, fibronectin, transforming growth factor-β_1, and -β_3 were significantly increased in the lungs of MI rats. The treatment with LU135252 did not alter this pattern of gene expression.

6 Thus, these data demonstrate pulmonary vascular remodelling and the increased expression of extracellular matrix proteins represent underlying mechanisms implicated in the development of pulmonary hypertension in the MI rat.

7 Despite the amelioration of the pulmonary hypertensive state, ET_A receptor blockade was insufficient to reverse pulmonary vascular remodelling, or the development of lung fibrosis in the MI rat.

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Abbreviations: ET-1, endothelin-1; ET_A, endothelin-A receptor; LU135253, (+)-(S)-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid; MI, myocardial infarction; PH, pulmonary hypertension; TGF-β, transforming growth factor-β

Introduction

A common pathophysiological feature of pulmonary hypertension (PH), regardless the etiology, is an increase in the circulating levels of endothelin-1 (ET-1) (Hay, 1999; MacLean, 1999; Stewart, 1993). In left ventricular failure, the most prevalent cause of secondary PH, elevated plasma ET-1 levels were found to relate directly with the severity of PH (Cody *et al*., 1992). Recent studies have confirmed the contribution of ET-1 in the development of left ventricular dysfunction and PH in the myocardial infarct (MI) rat model. The treatment with an ET receptor antagonist 1 week post-MI reduced mortality, improved cardiac function, and PH (Sakai *et al*., 1996a). By contrast, the early administration (24–48 h post-MI), did not have any beneficial effect or exacerbated the contractile dysfunction of the left ventricle, albeit an amelioration of PH was still evident (Sakai *et al*., 1996b; Nguyen *et al*., 1998). The apparent paradox with regard to the therapeutic efficacy of ET antagonists on left ventricular contractile function has been attributed to the suggested role of ET-1 in early scar formation and stabilization (Nguyen *et al*., 1998). Nonetheless, regardless of the time frame of ET-1 receptor antagonist administration, the beneficial effect on PH persists in the MI rat model.

The underlying mechanism(s) attributed to the beneficial effect of ET-1 receptor antagonist treatment on the development of PH in the MI rat remains unresolved. Pulmonary vascular remodelling and the progression of fibrosis represent pathophysiological events implicated in the genesis and/or maintenance of PH (Prié *et al*., 1997; Zhang & Phan, 1996). Indeed, several *in vivo* studies have shown the treatment with ET-1 receptor antagonists inhibited vascular smooth muscle hypertrophy in the DOCA-salt rat model of hypertension, and following angiotensin II infusion (Schiffrin, 1998; Moreau *et al*., 1997). Likewise, the administration of ET-1 receptor antagonists reduced the extent of fibrosis in the lungs of bleomycin-treated rats (Park *et al*., 1997). These latter data are

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consistent with the *in vitro* findings demonstrating the exogenous administration of ET-1 increased collagen levels in vascular smooth muscle cells, and fibroblasts (Rizvi and Myers, 1997; Guarda *et al*., 1993). Collectively, these observations suggest ET-1 receptor antagonists may improve PH *via* the modulation of vascular remodelling and the expression of extracellular matrix proteins. In this regard, the present study examined whether the development of PH in the MI rat was associated with pulmonary vascular remodelling, and a molecular phenotype of fibrosis. Secondly, the potential role of ET-1 in these pathophysiological events was examined *via* the administration of the ET_A receptor antagonist LU135252 24 h post-MI for a period of 4 weeks.

Methods

Experimental protocol

Myocardial infarction (MI) was induced in male Wistar rats (7 weeks old; 200–250 grams; Charles Rivers, St-Constant, Quebec, Canada) by ligating the left anterior coronary artery as previously described (Nguyen *et al*., 1998). The animals were randomized into four groups: (1) Sham-operated + 0.9% saline, (2) Sham-operated + LU135252 (Knoll AG), (3) MI rats + 0.9% saline, and (4) MI rats + LU135252. A dose of 60 mg kg^{-1} per day of LU135252 was administered twice daily by gavage 24 h following coronary-ligation or sham-operation for a period of 4 weeks. LU135252 is a novel nonpeptidic selective ET_A receptor antagonist with high oral bioavailability and a long half-life (Münter *et al*., 1996). This dose and regimen has been previously shown to inhibit the increased expression of lung ET-1 protein levels and myocardial ET-1 mRNA levels, and decrease right ventricular systolic pressure in the MI rat (Nguyen *et al*., 1998; Picard *et al*., 1998). Likewise, in the monocrotaline-induced model of pulmonary hypertension, the administration of 50 mg g^{-1} day^{-1} of LU135252 by gavage ameliorated right ventricular systolic pressure and hypertrophy (Prié *et al*., 1997).

In the present study, the criteria used to categorize a large MI is a scar to body weight ratio >0.2 mg g^{-1}, as previously described, and validated (Nguyen *et al*., 1998). The use and care of laboratory animals was according to the Canadian Council for Animal Care and approved by the Animal Care Committee of the Montreal Heart Institute.

Haemodynamic measurements

The haemodynamic profile of each rat was examined at 4 weeks post-infarction, as previously described (Nguyen *et al*., 1998). Briefly, rats were anaesthetized with a gas mixture containing 100% oxygen and halothane reduced from 2% to 0.5–0.8% 15 min before left and right heart haemodynamics were measured by a microtip pressure transducer catheter (model SPR-407, 2F, Millar instrument, Houston, Texas, U.S.A.).

Northern hybridization

Following haemodynamic measurements, total lung RNA was isolated by a modification of guanidine thiocyanate-phenol-chloroform extraction method, as previously described (Calderone *et al*., 1998). Total RNA (20 μg) was denatured with formaldehyde and formamide, and separated by size electrophoresis on a 1.3% agarose/4% formaldehyde gel, and subsequently transferred to nylon membranes (Genescreen Plus; Dupont-NEN) by vacuum blotting (model 785; Bio-Rad Laboratories).

A 0.985 kb fragment of rat TGF-β_1 (American Type Culture Collection (ATCC); Rockville, MD, U.S.A.), a 1.2 kb fragment of mouse TGF-β_3 (ATCC), a 0.6 kb fragment of rat fibronectin (courtesy of Dr R.O. Hynes), and a 0.3 kb fragment of prepro-collagen-α1 (type 1; ATCC) were labelled with ^{32}P-dCTP (NEN) to a specific activity of $1-2\times10^6$ c.p.m./ng cDNA by the random hexamer (Pharmacia) priming method and hybridized to nylon membranes (Dupont-NEN) for 18–24 h at 42°C, as previously described (Calderone *et al*., 1998). The filters exposed to the cDNA probes were washed twice (15 min, room temperature) with 300 mmol/L NaCl/30 mmol/L trisodium citrate and 0.1% SDS and twice (15 min, 45°C) with 30 mmol/L NaCl/3 mmol/L trisodium citrate and 0.1% SDS. Nylon membranes were subsequently exposed to Kodak XAR film with an intensifying screen at −70°C, and films were scanned with a laser densitometer (Chemilmager 4000 I v4.04 software; Alphan Innotech Corporation). All levels of mRNA reported in this paper are normalized to the level of 28S rRNA.

Lung vascular morphometry

The pattern of pulmonary arterial bed remodelling was examined in a separate series of experiments. The scar/bw ratio of the MI rats + 0.9% saline was 0.3 ± 0.03 ($n=7$), and the MI rats + LU135252 was 0.32 ± 0.03 ($n=5$). The pulmonary artery was injected at 50 cm H_2O pressure with a hot barium-gelatin mixture (60°C) for 3 min, and the airways were distended with 10% formaldehyde solution at a pressure of 36 cm H_2O.

The lungs were immersed in an inflated state in 10% formaldehyde solution for at least 2 days. For morphometric

Table 1 Morphology parameters of myocardial infarction rats

	Body wt, g	*Scar wt*, g	*Scar wt/Body* wt mg g^{-1}	*RV wt*, g	*RV wt/Body* wt mg g^{-1}	*LV wt*, g	*LV wt/Body* wt, mg g^{-1}	*Lung wt*, g	*Lung wt/Body* wt mg g^{-1}
Sham + saline ($n=14$)	437 ± 7	–	–	0.21 ± 0.01	0.48 ± 0.03	0.85 ± 0.02	1.93 ± 0.04	1.77 ± 0.08	4.05 ± 0.24
Sham + LU 135252 ($n=13$)	425 ± 11	–	–	0.21 ± 0.02	0.48 ± 0.04	0.87 ± 0.02	2.05 ± 0.06	1.76 ± 0.09	3.98 ± 0.19
MI + saline ($n=18$)	380 ± 7*	0.13 ± 0.01*	0.34 ± 0.02*	0.40 ± 0.01*	1.05 ± 0.05*	0.74 ± 0.03*	1.94 ± 0.0	2.99 ± 0.24*	7.64 ± 1.20*
MI + LU 135252 ($n=18$)	361 ± 7*	0.12 ± 0.01*	0.34 ± 0.01*	0.40 ± 0.02*	1.12 ± 0.06*	0.73 ± 0.02*	2.02 ± 0.04	3.08 ± 0.16*	8.52 ± 0.44*

Body wt, body weight; LV wt/body wt, left ventricular weight/body weight; RV wt/body wt, right ventricular weight/body weight; Lung wt/body weight, lung weight/body weight; Scar wt, scar weight; Scar wt/body wt, scar weight/body weight. Data presented are mean value ± s.e.mean. *$P<0.05$ vs sham.

analysis, three sections were obtained from each rat lung (from the upper, median and lower parts), stained with haematoxylin and eosin, and microscopically assessed for medial wall thickness of pulmonary arteries. The measurements of luminal diameter and medial thickness on either side (m_1 and m_2) were made along the shortest diameter. Measurements were made at random on 10 muscular arteries (size ranges 50–100, 101–150 and >150 μm in external diameter) per lung section. For each artery, the medial wall thickness was related to the external diameter (d, luminal diameter + media on either side) and expressed as per cent wall thickness (%WT) = $[(m_1 + m_2)/2d] \times 100$ (Prié *et al*., 1997).

Data analysis Data are represented as the mean ± s.e.m. Statistical analysis was performed by Student's unpaired *t*-test (2-tailed), and by analysis of variance followed by Dunnett's test when appropriate. A value of $P<0.05$ was considered statistically significant.

Results

Morphologic and haemodynamic alterations in the myocardium of MI rats: The effect of the ET$_A$ receptor antagonist LU135252

The ligation of the anterior descending coronary artery resulted in myocyte cell loss, as reflected by the significant decrease in left ventricular weight and scar formation in the MI group, as compared to sham (Table 1). A concomitant hypertrophic response was observed in the right ventricle, and lung wet weight was significantly increased in the MI rat group (Table 1). Left ventricular dysfunction was observed in the MI rat group, as reflected by the decrease in left ventricular systolic pressure, dP/dt index, and an increase in left ventricular end-diastolic pressure (Table 2). Moreover, PH was evident in the MI rat group, as reflected by the significant increase of right ventricular systolic pressure (Table 2). Long-term therapy of the MI rat group with the ET_A receptor antagonist LU135252 resulted in a significant reduction of right ventricular systolic pressure in the MI rats, without a concomitant change in ventricular hypertrophy, lung wet weight, scar size or left ventricular contractile function (Table 2).

Pulmonary artery remodelling in the MI rats

Morphometric changes of the pulmonary arteries are shown in Table 3. The external diameter of the caliber of arteries examined was similar in all sham and MI rats with or without LU135252 treatment. The luminal diameter of 50–100 μm pulmonary arteries was significantly decreased in the MI rats, as compared to sham. By contrast, in the 101–150 and 151–200 μm caliber arteries, a non-significant decrease in luminal diameter was observed in the MI rats, as compared to sham. The treatment with LU135252 did not reverse the decrease in luminal diameter observed in the MI rats. Medial wall thickness was significantly increased in all the caliber of arteries examined in the MI rats, as compared to sham, regardless the arterial caliber. LU135252 treatment did not reverse this pattern of remodelling.

A molecular phenotype of pulmonary fibrosis

To assess whether the MI rats were associated with the development of lung fibrosis, the steady-state mRNA levels of the extracellular matrix proteins collagen and fibronectin were assessed. In the lungs of the MI rats, the steady-state mRNA levels of prepro-collagen-α1 and fibronectin were increased 3.7 ± 1.0 fold ($P<0.05$ versus sham; $n=6$) and 2.9 ± 0.2 fold ($P<0.05$ versus sham; $n=6$) respectively, in the lungs of MI rats, as compared to sham rats (Figure 1). The transforming growth factor-β (TGF-β) family has been implicated as a local autocrine/paracrine factor involved in the progression and/or maintenance of fibrosis. Consistent with this premise, the increased mRNA expression of the extracellular matrix proteins was associated with a concomitant significant increase in the steady-state mRNA levels

Table 2 Haemodynamic parameters 4 weeks after infarction

	RVSP mm Hg	*RVEDP* mm Hg	*RV dP dt^{-1}* mm Hg s^{-1}	*LVSP* mm Hg	*LVEDP* mm Hg	*LV dP dt^{-1}* mm Hg s^{-1}
Sham + saline ($n=8$)	26.8 ± 1.3	3.4 ± 0.9	1178 ± 86	108 ± 2.4	6.5 ± 0.9	4987 ± 334
Sham + LU 135252 ($n=7$)	26.3 ± 0.56	2.3 ± 0.9	1237 ± 91	108 ± 4.4	5.9 ± 1.3	4550 ± 488
MI + saline ($n=11$)	52.4 ± 3.2*	14.7 ± 2.3*	1394 ± 64	85 ± 3*	26 ± 1.5*	2625 ± 251*
MI + LU 135252 ($n=13$)	43.6 ± 1.8*#	13.4 ± 1*	1290 ± 80	88 ± 3*	29 ± 1.2*	2529 ± 109*

RVSP, right ventricular systolic pressure; RVEDP, right ventricular end diastolic pressure; RV dP/dt, right ventricular dP/dt; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; LV dP/dt, left ventricular dP/dt. Values are means ± s.e.mean. *$P<0.05$ vs sham. #$P<0.05$ vs MI + saline.

Table 3 Effects of LU 135252 on myocardial infarction-induced morphometric changes in pulmonary arteries

	Luminal diameter (μm)			*External diameter* (μm)			*Medial wall thickness* (%)		
	50–100 μm	101–150 μm	151–200 μm	50–100 μm	101–150 μm	151–200 μm	50–100 μm	101–150 μm	151–200 μm
Sham + saline ($n=6$)	60.4 ± 1.5	99.5 ± 2.9	160.6 ± 10	78.5 ± 1.5	123.1 ± 2.3	196.1 ± 10.5	11.8 ± 0.5	9.7 ± 0.9	8.9 ± 1.6
Sham + LU 135252 ($n=6$)	59.9 ± 1.5	96.9 ± 3.1	139.8 ± 10	81.8 ± 1.5	128.9 ± 2.4	189.8 ± 10.5	13.5 ± 0.5	12.4 ± 0.9	11.5 ± 1.6
MI + saline ($n=7$)	53.3 ± 2.0**	97.1 ± 4.5	134.3 ± 12.5	76.0 ± 2.0	131.7 ± 3.5	188.1 ± 13	15.0 ± 0.7**	13.3 ± 1.4*	14.7 ± 2.0*
MI + LU 135252 ($n=5$)	55.2 ± 1.7*	88.7 ± 2.8*	122.5 ± 9.3*	76.6 ± 1.6	122.9 ± 2.1	181.9 ± 9.7	14.2 ± 0.6*	13.9 ± 0.8**	16.4 ± 1.5**

*$P<0.05$; **$P<0.01$ vs sham; Values are mean ± s.d.

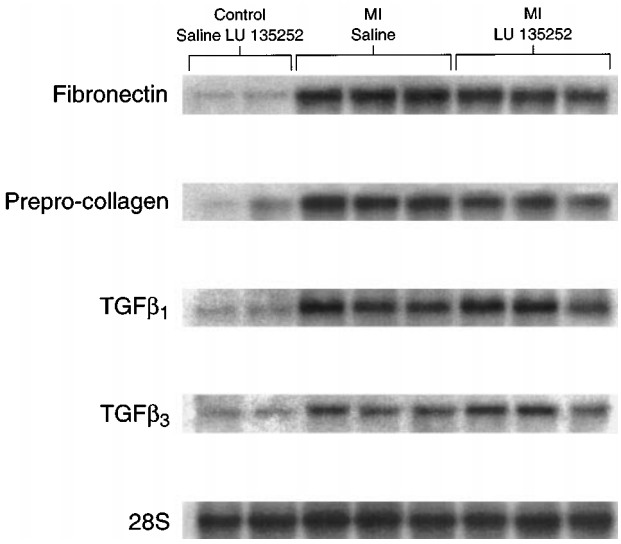

Figure 1 A Northern hybridization depicting changes in the steady-state mRNA levels of prepro-collagen α_1, fibronectin, transforming growth factor-β_1 (TGF-β_1), and TGF-β_3 in the total RNA isolated from rat lungs. The pattern of gene expression in the sham-operated rats treated with the ET$_A$ receptor antagonist LU135252 was similar to the sham-operated rats administered saline. The steady-state mRNA levels of prepro-collagen α_1, fibronectin, TGF-β_1, and TGF-β_3 were increased in the lungs of rats with myocardial infarction (MI). The long-term therapy with LU135252 did not reverse the pattern of gene expression in the MI rats. mRNA levels were normalized to the level of 28S ribosomal RNA.

of TGF-β_1 (3.7 ± 0.8 fold increase; $P<0.05$ versus sham; $n=6$) and TGF-β_3 (2.7 ± 0.7 fold increase; $P<0.05$ versus sham; $n=6$) in the lungs of the MI rats, as compared to the sham rats (Figure 1). Long-term therapy of sham rats with the ET$_A$ receptor antagonist LU135252 did not alter the expression of these transcripts, as compared to sham (Figure 1). In the MI rat group, LU135252 treatment did not prevent the increased expression of either collagen (3.0 ± 0.8 fold increase; $P<0.05$ versus sham + LU135252; $n=6$), fibronectin (2.4 ± 0.6 fold increase; $P<0.05$ versus sham + LU135252; $n=6$), TGF-β_1 (4 ± 0.8 fold increase; $P<0.05$ versus sham + LU135252; $n=6$), or TGF-β_3 (3.2 ± 0.8 fold increase; $P<0.05$ versus sham + LU135252; $n=6$) mRNAs, as compared to untreated MI rats (Figure 1).

Discussion

The development of PH, secondary to MI in the rat, was associated with pulmonary artery remodelling, and a pattern of gene expression consistent with the presence of fibrosis. The long-term administration of the ET$_A$ receptor antagonist LU135252 did not reverse these phenotypic events in the lungs, despite a significant reduction of right ventricular systolic pressure. Collectively, these data suggest ET-1, acting at least partially *via* the ET$_A$ receptor, may in part contribute to the development of PH. However, the absence of a beneficial effect of LU135252 on either pulmonary vascular remodelling or the fibrotic pattern of gene expression in the lungs of the MI rat, suggests ET-1 activation of the ET$_A$ receptor was not implicated in the progression of these pathophysiological events.

Data from several studies support the concept that ET-1, acting *via* the ET$_A$ receptor, is implicated in the development of PH. Evidence to support this premise includes increased expression of ET-1 in the lungs of various models of PH, and the identification of ET$_A$ receptors on the pulmonary arterial vasculature (Soma *et al*., 1999; Nguyen *et al*., 1998; Park *et al*., 1997; Sakai *et al*., 1996b; Nakamichi *et al*., 1992). Consistent with this concept, the administration of structurally distinct ET$_A$ receptor antagonists were shown to reverse the increase in pulmonary artery pressure, and the associated hypertrophic response of the right ventricle in hypoxia- and monocrotaline-induced models of PH (Prié *et al*., 1997; DiCarlo *et al*., 1995). In the lungs of the MI rat, ET-1 peptide levels were found elevated, and the long-term treatment with LU135252 completely suppressed the increased expression (Sakai *et al*., 1996b; Nguyen *et al*., 1998). These latter findings are consistent with a role of the ET$_A$ receptor in the synthesis of endothelin-1 (Barton *et al*., 1998). Moreover, coincident with LU135252 treatment, a significant reduction in right ventricular systolic pressure was observed in the MI rats. Likewise, the treatment of MI rats with the structurally distinct ET$_A$ receptor antagonist BQ-123 was found to ameliorate right ventricular systolic pressure when administered 24 h post-infarction (Sakai, 1996b). By contrast, neither LU135252 nor BQ-123 treatment ameliorated left ventricular contractile indices, or attenuated scar size in the MI rats (Sakai, 1996b). Collectively, these data suggest that ET-1 may have a pathophysiological role in the development of PH in the MI rat.

Although both LU135252 and BQ-123 ameliorated right ventricular systolic pressure in the MI, BQ-123 rather than LU135252 caused a significant decrease in right ventricular hypertrophy in the MI rats (Sakai *et al*., 1996b). The reason for this dichotomy between two ET$_A$ antagonists on right ventricular hypertrophy in the MI rat remains presently unknown. An important caveat which may in part explain these latter findings is that the reduction of right ventricular pressures with BQ-123 was greater than LU135252, and in this regard, the decrease by LU135252 may not have been sufficient to have any measurable effect on the hypertrophic response. This difference may be in part explained by the pharmacological profiles of these two antagonists. It has been demonstrated BQ-123 has a greater selectivity (500 – 2000 fold) for the ET$_A$ receptor versus the ET$_B$ receptor (Battistini & Dussault, 1998). By contrast, LU135252 has a 140 fold greater selectivity for ET$_A$ than ET$_B$ (Battistini & Dussault, 1998). Consequently, LU135252 treatment may have elicited some antagonism of the ET$_B$ receptor sufficient to counteract the beneficial effect of exclusively blocking ET$_A$ receptors. The ET$_B$ receptor is found predominantly on endothelial cells, and is coupled to the synthesis of nitric oxide and prostacyclin, two important vasodilatory substances (Love & McMurray, 1996). Thus, any antagonism of the ET$_B$ receptor by LU135252 would diminish the beneficial vasodilatory effect, thereby partially offsetting the decrease in right ventricular pressures mediated by ET$_A$ antagonism leading to less effective diminution of right ventricular hypertrophy. Moreover, nitric oxide has been shown to exert an antihypertrophic action on cardiac myocyte growth (Calderone *et al*., 1998). Thus, the concomitant blockade of endothelial ET$_B$ receptors in the myocardium could diminish the local growth-inhibiting action of nitric oxide, an effect less feasible with the highly selective ET$_A$ antagonist (Battistini & Dussault, 1998). Nonetheless, despite this difference, the ability of two structurally distinct ET$_A$ receptor antagonists (LU135252 and BQ-123) to ameliorate right ventricular systolic pressure supports the involvement of ET-1 in the development of PH in the MI rat.

It has been suggested that endothelin-1 may be involved in the development of pulmonary and systemic hypertension, and subsequent vascular bed remodelling. In the DOCA-salt rat model of hypertension, ET$_A$ receptor blockade markedly

reduced elevated blood pressure, and improved vascular remodelling (Schiffrin, 1998). Likewise, in an angiotensin II-infused experimental rat model of hypertension, the treatment with the ET_A antagonist LU135252 reduced blood pressure and attenuated vascular hypertrophy (Moreau *et al*., 1997). In the hypoxia-induced model of PH, BQ-123 treatment reversed the increase in pulmonary artery pressure and medial wall thickness of 50–100 μm caliber pulmonary arteries (DiCarlo *et al*., 1995). Consistent with these *in vivo* findings, the exogenous administration of ET-1 to cultured vascular smooth muscles cells promoted proliferation and hypertrophy *via* the recruitment of the small GTP-binding protein ras (Hafizi *et al*., 1999; Schieffer *et al*., 1997). Interestingly, in human vascular smooth muscle cells, ET-1 had no direct effect on growth, but potentiated the growth response to platelet-derived growth factor (Yang *et al*., 1999). This latter effect was blocked by the ET_A receptor antagonist LU135252. In the present study, the development of PH in the MI rat was associated with the concomitant remodelling of the pulmonary arterial bed. A significant decrease in luminal diameter in the 50–100 caliber arteries was observed, whereas a similar tendency, but non-significant effect was observed in the 101–150 and 151–200 μm caliber arteries. In all groups of caliber arteries examined, medial wall thickness was significantly increased in the MI rat, as compared to the sham-operated rat. However, despite the beneficial effect on right ventricular systolic pressure, pulmonary vascular remodelling was not attenuated in the MI rat following LU135252 therapy. Likewise, in the monocrotaline-induced model of PH, LU135252 treatment improved right ventricular systolic pressure, but did not reverse pulmonary artery remodelling (Prié *et al*., 1997). Thus, it is possible that alternative vascular smooth muscle mitogens, such as angiotensin II, and platelet-derived growth factor may be instrumental in pulmonary artery remodelling in the MI rat model, as well as the monocrotaline rat model of PH (Yang *et al*., 1999; Moreau *et al*., 1997).

Lung fibrosis represent a common pathophysiological feature of various pulmonary diseases (Kovacs & DiPietro, 1994), and both *in vivo* and *in vitro* studies have characterized ET-1 as a profibrotic factor. In the bleomycin-induced rat model of pulmonary fibrosis, immunoreactive ET-1 levels were increased, and the treatment with the non-selective ET receptor antagonist bosentan, reduced the extent of fibrosis (Park *et al*., 1997). In vascular smooth muscle cells, and fibroblasts, ET-1 administration led to the synthesis of collagen, and further decreased collagenase activity in fibroblasts (Rizvi & Myers, 1997; Guarda *et al*., 1993). Lastly, in pulmonary bronchial epithelial cells, and cultured rabbit synoviocytes, ET-1 increased fibronectin gene expression *via* activation of the ET_A receptor (Gutierrez *et al*., 1996; Marini *et al*., 1996). In the present study, the presence of PH in the MI rat was associated with an increase in the steady-state lung mRNA levels of collagen and fibronectin. The long-term therapy with LU135252 did not reverse the increased expression of these extracellular matrix proteins. Consequently, these data are, to the best of our knowledge, the first to highlight a molecular phenotype of fibrosis in the lungs of the MI rat, which may in part contribute to the development of PH. The inability of LU135252 to block this molecular fibrotic phenotype suggests the ET_A receptor is not involved in this process, at least in the lungs of the MI rat. By contrast, it is possible that there may exist a certain degree of redundancy in this process, such that despite the blockade of the ET_A receptor, alternative local and/or hormonal factors may initiate the development of extracellular matrix remodelling.

In vitro studies have demonstrated that the exogenous administration of the transforming growth factor-β (TGF-β) isoforms to lung fibroblasts increased collagen expression, with TGF-β_3 the most potent profibrotic agent (Eickelberg *et al*., 1999; Coker *et al*., 1997). Consistent with the *in vitro* effects of TGF-β, an increased expression of TGF-β isoforms, as well as a direct role of TGF-β_1 in the development of lung fibrosis has been documented (Nakao *et al*., 1999; Coker *et al*., 1997; Sime *et al*., 1997). In the lungs of the MI rats, the steady-state mRNA levels of TGF-β_1 and TGF-β_3 were increased, as compared to sham rats. The long-term therapy of MI rats with LU135252 did not prevent the increased expression of either TGF-β_1 or TGF-β_3 mRNA, thereby suggesting ET-1 activation of the ET_a receptor was not involved in their regulation. Nonetheless, the concomitant increased mRNA expression of extracellular matrix proteins and TGF-β isoforms is consistent with a role of these peptide growth factors in the progression and/or maintenance of lung fibrosis in the MI rat. The cellular origin of the increased expression of the TGF-β isoforms, and the hormonal and/or local factors implicated in their expression remain to be determined.

In conclusion, the long-term therapy of MI rats with the ET_A selective antagonist LU135252 caused a significant reduction in right ventricular systolic pressure, suggesting ET-1, acting at least partially *via* the ET_A receptor, contributes in part to the development of PH. However, neither pulmonary artery remodelling nor the increased expression of lung collagen and fibronectin mRNAs were prevented by long-term LU135252 treatment in the MI rat. Lastly, the profibrotic agents, TGF-β_1 and TGF-β_3 mRNAs were increased in the MI rats, and these peptide growth factors may play an instrumental role in the progression and/or maintenance of fibrosis in the lung of MI rats.

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