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Review Article

Sitaxsentan: update on basic science studies and clinical trials supporting selective endothelin ET_A receptor antagonism in the treatment of pulmonary arterial hypertension

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CONTENTS

Abstract

Pulmonary arterial hypertension (PAH) is a rare and typically progressive condition characterized clinically by sustained increases in mean pulmonary artery pressure and pulmonary vascular resistance, which often culminate in fatal right heart failure. The disease is characterized histopathologically by vascular remodeling, with neointimal formation, fibrosis and vessel wall thickening of small pulmonary arteries. Treatment options are limited, with only one oral endothelin receptor antagonist (ETRA) therapy being approved to date for use in the United States. The highly selective endothelin ET_A receptor antagonist sitaxsentan represents a promising therapy for this frequently fatal condition.

Introduction

Background on pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a rare, life-threatening disease characterized by progressive narrowing of the distal precapillary pulmonary vasculature, with vessel wall thickening and intimal proliferation in small, muscular arteries. In the older literature, PAH is often termed pulmonary hypertension (PH). This terminology is misleading, because elevated pulmonary artery pressure (PAP) can result passively from elevated pulmonary venous pressures due to left-sided heart disease (*e.g.*, ischemic heart disease, valvular disease). These left-sided heart diseases cause elevated PAP more frequently than PAH, but do not respond to PAH therapies.

The natural history of PAH is marked by progressive vasoconstriction and pulmonary vascular remodeling, culminating in right heart failure and death. Under normal circumstances, pulmonary vascular beds offer less than 10% of the resistance to flow offered by the systemic vasculature (1). The hemodynamic hallmark of PAH is a mean PAP of 25 mmHg or more at rest (30 mmHg or more upon exertion), with a mean pulmonary capillary wedge pressure (PCWP) or left ventricular end-diastolic pressure (LVEDP) of 15 mmHg or less and pulmonary vascular resistance (PVR) of 3 mmHg/l/min or more.

Pulmonary arterial hypertension often has an insidious onset, with initial clinical manifestations that include dyspnea (shortness of breath), fatigue and reduced exercise tolerance in patients aged 20 through 40 years. The causes of these manifestations may initially be unclear or nonspecific. Dyspnea upon exertion is among the earliest manifestations of PAH. Lower extremity edema secondary to venous congestion may develop with the onset of right ventricular failure. Angina is also a common symptom, and syncope or near syncope may result as cardiac output declines. In a national registry of patients with idiopathic PAH, the mean age was 36 years, and the mean time from symptom onset to diagnosis was 2 years (2). A later publication from the same registry reported a median survival of 2.8 years (3).

According to the most recent consensus nomenclature (4), PAH is categorized as either idiopathic, familial or secondary to a number of conditions, including collagen vascular disease (*e.g.*, scleroderma), congenital systemic-to-pulmonary shunts, human immunodeficiency virus (HIV) infection, portal hypertension and exposure to drugs/toxins, including certain anorexigens (appetite suppressants) (5), such as fenfluramine and aminorex. Approximately 87,000 North Americans and Europeans have clinically advanced PAH (1).

Familial PAH is an autosomal dominant disease with a female:male ratio of 1.7:1 and exhibits genetic anticipation, such that the disease worsens in younger generations. In 1997, two separate groups mapped a *PPH1* gene to a region of chromosome 2q31-q32 (6, 7). More recent work determined that familial PAH involves mutations in the gene for bone morphogenetic protein receptor 2 (*BMPR2*) (8-10), a member of the transforming growth factor-β (TGF-β) superfamily of proteins, which regulate tissue growth and repair (11). Although familial PAH accounts for a small minority of all cases of PAH, a recent (controversial) hypothesis is that defects in the BMPR2 and other signaling pathways (*e.g.*, angiopoietin-1) underlie both familial and nonfamilial forms of PAH (12).

Pulmonary arterial hypertension associated with scleroderma has a poor prognosis (13). Frequently termed systemic sclerosis, scleroderma may manifest as diffuse cutaneous disease, with symmetric skin thickening of the face, trunk and both proximal and distal extremities, or limited cutaneous disease, with symmetric skin thickening of the face and distal extremities only. The latter subset frequently involves features of the CREST syndrome: *c*alcinosis, *Ray*naud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly and *t*elangiectasia. Up to half of the patients with CREST syndrome develop PAH. Although mixed connective tissue disease is less common than scleroderma, up to two-thirds of patients with this condition develop PAH (1).

Other causes of increased blood pressure within the pulmonary circulation include pulmonary venous hypertension, which may be secondary to left ventricular disease (*e.g.*, heart failure) or left-sided valvular disease; chronic thromboembolic disease, including obstruction of the distal pulmonary arteries via pulmonary embolism or sickle cell disease (14); disorders of the respiratory system and/or hypoxemia, including chronic obstructive pulmonary disease (COPD), sleep-disordered breathing and interstitial lung disease; and diseases directly affecting the pulmonary vasculature, including inflammatory conditions such as schistosomiasis and sarcoidosis (4).

Pulmonary arterial hypertension is associated with vascular cell dysfunction, with imbalances in the formation or expression of a wide range of vasoactive mediators and cell proteins (15, 16). These imbalances include reduced formation of vasodilators, such as nitric oxide (NO) (17, 18), prostacyclin (PGI_2) (19, 20) and vasoactive intestinal peptide (VIP) (21), increased formation of vasoconstrictors, such as endothelin-1 (ET-1), angiotensin II and serotonin (5-hydroxytryptamine [5-HT]) (22-27), as well as increased production of the prothrombotic arachidonic acid metabolite thromboxane A_2 (TxA_2) (19, 28). Although initially conceived as a manifestation of endothelial cell dysfunction, PAH is now increasingly being construed pathophysiologically as a disease affecting other cells in the vessel wall, including smooth muscle cells and fibroblasts. This paradigm shift has resulted, in part, from the findings of mutations in the *BMPR2* gene within pulmonary artery smooth muscle cells isolated from patients with PAH (29).

Another molecular mechanism for vasoconstriction in PAH is via alterations in transmembrane ion channels and transporters. For instance, the expression and function of certain types of potassium channels may be reduced in patients with PAH or in experimental models of PAH (30-32). The resultant depolarization of the vascular smooth muscle cell enables increased entry of calcium (Ca^{2+}), with increased actomyosin bridge formation and attendant smooth muscle cell contraction (vasoconstriction). Increased formation of the 5-HT transporter (SERT) (33) may also contribute to PAH pathogenesis. In summary, the multiplicity of signaling pathways implicated in the pathogenesis of PAH opens numerous lines of potential pharmacological attack, with the promise of more effective therapies.

At the pathological level, hypertensive pulmonary arteriopathy is present in 85% of cases of idiopathic PAH, with medial hypertrophy of the arteries and arterioles frequently in tandem with other vascular changes, particularly intimal proliferation (1). Intimal proliferation may manifest with either concentric laminar (or nonlaminar) intimal fibrosis or with eccentric intimal fibrosis, and may be occlusive.

Plexiform lesions are an important feature of disease progression in all forms of PAH. Once believed to be found exclusively in patients with idiopathic PAH, plexiform lesions have also been identified in patients with PAH associated with collagen vascular disease and other forms of secondary PAH (34, 35), and in animal models of PAH (36). Plexiform lesions and a necrotizing arteritis may develop throughout the lungs. Plexiform lesions result from "exuberant" and disordered growth of endothelial cells and smooth muscle cells to form a glomeruloid structure with anastomosing channels in the branches of the pulmonary artery, under the influence of growth factors and cytokines (37). Immunohistochemically and morphologically, these lesions are not dissimilar to the neovascularization observed in glioblastoma multiforme (37). Because these lesions express markers of angiogenesis (*e.g.*, vascular endothelial growth factor receptor, or VEGFR), they may represent a form of disordered angiogenesis (38). Plexiform lesions may result in aneurysmal dilation and/or complete obstruction of pulmonary vessels. Also observable in some cases of idiopathic PAH is widespread *in situ* thrombosis, with intraluminal deposits of thrombin in small pulmonary arteries (1).

Disordered angiogenesis constitutes a key factor underlying the pathogenesis of small vessel obstructive

vasculopathy in PAH. In this context, Benisty *et al.* demonstrated that an angiogenic growth factor (basic fibroblast growth factor, or bFGF) was significantly increased in the urine and plasma of 117 patients with PAH as compared with 60 control subjects, with growth factor levels being particularly high in patients with idiopathic PAH (39). Furthermore, Tuder's group showed that endothelial cells within plexiform lesions of patients with severe PAH expressed VEGF and overexpressed the mRNA and protein of VEGFR-2, as well as transcription factors responsible for hypoxia-dependent induction of VEGF (38).

Right heart catheterization remains the "gold standard" for the diagnosis of PAH. However, other diagnostic modalities may also contribute important information. In addition to symptoms such as dyspnea on exertion, weakness or fatigue, chest pain, syncope, abdominal distension and peripheral edema, four modalities are central to detect PAH: physical examination, chest radiography, electrocardiography (ECG) and echocardiography (*e.g.*, transthoracic Doppler echocardiography [TTE]). The diastolic murmur of pulmonary insufficiency or the pansystolic murmur of tricuspid regurgitation may be discernible on auscultation. Other potentially suggestive symptoms and signs include peripheral edema, hepatomegaly, ascites, jugular vein distension and cool extremities (40).

Enlargement of the pulmonary artery and/or right ventricle on chest radiography also suggests the presence of PAH (40). Other roentgenographic findings may be suggestive of certain causes of pulmonary hypertension, including pronounced asymmetry of enlarged central pulmonary arteries in the presence of chronic thromboembolic disease, and hyperinflation in the presence of obstructive pulmonary disease.

Although the ECG alone is considered inadequate as a screening modality for significant PAH, it may offer supportive evidence by demonstrating right atrial dilation or right ventricular hypertrophy (40). There is a high correlation between measurements of pulmonary arterial systolic pressure by TTE and right heart catheterization. In experienced hands, quantifiable tricuspid regurgitant signals are detected on Doppler ultrasonography in up to 74% of cases of PAH (41).

Additional tests are used to characterize PAH (40). Essential tests to determine severity and prognosis, choose therapy or characterize potential substrates include pulmonary function testing; screening overnight oximetry to rule out sleep apnea or hypopnea; ventilation-perfusion V/Q lung scintigraphy to identify possible chronic thromboembolic pulmonary hypertension; blood tests, including complete blood count (with platelet count), antinuclear antibody to screen for collagen vascular disease, antiphospholipid antibodies and HIV serology; assessment of exercise capacity, including the 6-min walk (6MW) test, cardiopulmonary exercise testing and exercise Doppler echocardiography; and right heart catheterization with acute vasodilator testing. Patients with a decrease in mean PAP of 10 mmHg or more (to 40 mmHg or less) in response to vasodilators without diminished cardiac output may benefit from therapy with calcium channel blockers. Unfortunately, a small minority (~10%) of patients with PAH exhibit such responses.

Contingent testing, which is dependent on the presentation and outcomes of essential testing and is not necessary in all patients, includes transesophageal echocardiography (TEE), which may be useful to detect chronic thromboemboli and intracardiac shunts (*e.g.*, atrial septal defects); chest computerized tomography (CT), spiral or helical CT, electron-beam CT or pulmonary angiography to identify chronic thromboembolic disease in the presence of an abnormal V/Q scan; high-resolution CT to identify pulmonary capillary hemangiomatosis or pulmonary veno-occlusive disease; coagulation studies, particularly if chronic thromboembolic pulmonary hypertension is suspected or known; additional blood tests, including arterial blood gas or oximetry measurements, uric acid levels and brain natriuretic peptide (BNP); and polysomnography if screening overnight oximetry testing or the history is suggestive (40).

Therapies for PAH are limited. In the majority of patients, who do not exhibit positive responses to calcium channel blockers, treatment may consist of the nonselective endothelin receptor antagonist (ETRA) bosentan, which is approximately 20 times more selective for ET_A receptors compared with ET_B receptors, or prostacyclin analogues, which have short plasma residence times and hence require continuous or frequent administration. Other approved therapies include the phosphodiesterase type 5 (PDE5) inhibitor sildenafil and inhaled NO.

Bosentan is the only approved oral ETRA therapy for PAH. Candidates for treatment with this nonselective ETRA include patients with PAH functional class III who are not eligible for, or have not responded to, calcium channel blockade (42). Also available for these patients and those with more severe disease (functional class IV) are parenteral and inhaled prostacyclin analogues, including intravenous (i.v.) epoprostenol, i.v. and subcutaneous (s.c.) treprostinil, and inhaled iloprost (42). Treatment with the PDE5 inhibitor sildenafil, which was recently approved, may be considered in patients with World Health Organization (WHO) group I and functional class II-IV PAH (42, 43). Adjunctive treatments with agents having distinct and potentially complementary mechanisms of action have also been evaluated in clinical trials (44, 45).

Background on ET-1 and the case for selective ET_A receptor antagonism

Despite its diverse pathogenesis, PAH is characterized by two cardinal features: vasoconstriction and vascular remodeling. On the basis of both preclinical and clinical evidence, ET-1 is considered to be a key mediator of both of these processes. A vascular cell mitogen with profibrotic properties, ET-1 is the predominant isoform in a family of three 21-amino-acid peptides that was isolated from bovine pulmonary and aortic endothelium and whose gene sequence was identified in 1987 (46-

52). Yanagisawa *et al.* isolated ET-1 from porcine cells in 1988 and characterized it as one of the most potent endogenous vasoconstrictors identified to date (50).

Endothelin-1 elicits long-lasting vasoconstrictor effects in part by influencing voltage-dependent ion channels and calcium mobilization via dihydropyridine-sensitive calcium channels. Some of these effects are mediated via ET-1's ability to activate phospholipase C (PLC), which promotes accumulation of inositol triphosphate, diacylglycerol and intracellular calcium in vascular smooth muscle cells (53-56). Endothelin causes hypertension in laboratory animals and humans, and constricts coronary arteries *in vitro* (57). Preclinical evidence suggests that alterations in ET-1 signaling constitute the earliest pathological defect in experimental models of PAH, preceding changes in NO signaling (58).

Clinical evidence supporting the role of ET-1 in the pathogenesis of PAH includes findings of increased circulating levels of ET-1, as well as localization of increased ET-1 to the vascular endothelium of arteries showing extensive vascular remodeling in PAH, with intimal fibrosis and medial thickening (59-61). In a study in patients with idiopathic PAH, levels of both the ET-1 precursor big ET-1 and the mature ET-1 peptide correlated strongly with both mean PAP and PVR, as well as with various indices of PAH severity (*e.g.*, limitations in 6MW distance) (62). The finding of high ET-1 levels in the pulmonary vein was consistent with increased synthesis and release of ET-1 by the lung in patients with idiopathic PAH. Approximately 50% of circulating ET-1 is cleared with each passage of blood through the lungs (63).

The physiological effects of ET-1 depend largely on interactions with two populations of receptors: ET_A and ET_B. Endothelin receptor expression is increased in animal models of PAH and in humans with this condition. ET_B receptor expression may be increased in some patients with PAH, although no functional role for this receptor in the disease process has been identified. It is possible that the increase in ET_B receptor expression represents a compensatory mechanism aimed at clearing the increased ET-1 peptide that occurs in PAH patients. ET_B receptors modulate the proliferative characteristics of pulmonary artery smooth muscle cells, as demonstrated in a cell culture assay system in which human pulmonary artery smooth muscle cells were grown on plastic in an artificial environment devoid of blood flow, matrix or innervations (64).

Binding of ET-1 to ET_A receptors on vascular smooth muscle cells promotes chronic vasoconstriction in part by activating the PLC pathway. ET_A receptor activation also stimulates smooth muscle cell proliferation (hyperplasia) and hypertrophy underlying vessel wall thickening of small, muscular pulmonary arteries (65-70). Endothelin stimulates smooth muscle cell hyperplasia in part via increased expression of proto-oncogenes and in cooperation with co-mitogens such as TGF-α, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) (69-72).

The ET-1/ET_A receptor signaling pathway is also considered central to the profibrotic effects of ET-1. Via ET_A receptor-dependent mechanisms, ET-1 induces expression of extracellular matrix proteins, including collagen and fibronectin (73-76), by fibroblasts and other cells, as well as promoting the ability of fibroblasts to contract extracellular matrix (77, 78).

Binding of ET-1 to endothelial cell ET_B receptors may help to counter the pathophysiology of PAH by helping to clear ET-1 via the lung (79, 80) and kidney (81), as well as by promoting vasodilation (82) and reuptake of ET-1 by endothelial cells (83). The ET-1/ET_B receptor signaling pathway also inhibits endothelin-converting enzyme (ECE), thus assisting in reducing ET-1 formation (46, 84). In addition, the ET_B receptor is a Gi protein-coupled receptor that is associated with both NO synthase (NOS) and cyclooxygenase (COX). Through these interactions, binding of ET-1 to ET_B receptors transduces the synthesis and release of NO and PGI_2, substances which exert powerful vasodilating, antiproliferative and antiplatelet effects.

It appears that ET-1/ET_A receptor signaling pathways promote smooth muscle hyperplasia and fibrosis in pulmonary vascular remodeling, whereas ET-1/ET_B receptor pathways promote ET-1 clearance and vasodilator synthesis by endothelial cells. Therefore, selective ET_A receptor antagonism might be expected to block the deleterious vasoconstrictor and vasoproliferative effects of smooth muscle cell ET_A receptors while preserving salutary functions mediated by endothelial cell ET_B receptors. As with the debate concerning selective compared with nonselective β-adrenoceptor blockers, the argument that selective ET_A receptor blockade is preferable to nonselective ET_A/ET_B receptor blockade can be adjudicated only via head-to-head comparative clinical trials. However, both preclinical and clinical evidence seems to support the desirability of selective ET_A receptor antagonism.

Preclinical evidence includes findings in rodents deficient in the gene encoding the ET_B receptor. These organisms are predisposed to develop severe PAH, with accelerated neointimal formation, increased PVR, vasoconstriction with diminished filling of small pulmonary arteries (lower arterial-to-alveolar ratio), decreased levels of the vasodilators NO (and NOS) and PGI_2, as well as increased blood pressure and ET-1 levels compared to rats having normal ET_B receptor expression (36, 85-87). The ET_B receptor appears to confer protective effects in hypoxia- and monocrotaline-induced PAH. In a recent report by Ivy's group (36), morphometric evaluation demonstrated neointimal proliferation in small distal pulmonary arteries among 60% of ET_B receptor–deficient rats upon exposure to the endothelial toxin monocrotaline. Among these animals, barium angiography showed reduced filling of small pulmonary arteries, and this decreased arterial-to-alveolar ratio was consistent with the development of occlusive neointimal lesions within these arteries. These arteries were characterized not only by neointimal lesions, but also by thickening of the medial wall and increased expression of the proangiogenic VEGF within neointimal lesions (36). In addition to

VEGF, high levels of tenascin-C, another important proangiogenic molecule, were observed. Furthermore, ET_B deficiency predisposes rats to pulmonary edema formation in a VEGF-dependent manner (88).

Beneficial effects of selective ET_A receptor antagonism have been observed in both healthy volunteers and patients with various manifestations of endothelial dysfunction, including heart failure, chronic renal failure and atherosclerosis (89-91). Verhaar *et al.* evaluated brachial artery (forearm) blood flow in 22 healthy subjects aged 20-43 years (89). Forearm blood flow is a practical index of endothelial function in human resistance arteries. Infusion of the ET_A receptor antagonist BQ-123 was associated with significant, progressive increases in forearm blood flow, which reached a plateau at 60 min. The vasodilator response to ET_A receptor blockade was blunted during "NO clamping" using the NOS inhibitor L-N^G-monomethyl-arginine (L-NMMA). This finding supports the premise that an intact ET-1/ET_B receptor pathway with ongoing ET_B receptor-mediated NO synthesis contributed to the vasodilation observed during selective ET_A receptor blockade.

However, infusion of the selective ET_B receptor antagonist BQ-788 alone was associated with vasoconstrictor effects, with a small but consistent 20% reduction in forearm blood flow through 120 min. In addition, the vasodilator effects of the selective ET_A receptor blocker BQ-123 were blunted in the presence of the selective ET_B receptor blocker BQ-788: forearm blood flow increased 47% at 120 min during concomitant BQ-123 and BQ-788 infusion as compared with an increase of 76% when BQ-123 was administered alone ($p < 0.001$) (89).

Further evidence for the potential vasoconstrictor effects of selective ET_B receptor blockade derives from a study by Love's group of forearm blood flow in 10 healthy subjects and 10 patients with chronic heart failure (CHF) (90). Administration of the selective ET_A receptor blocker BQ-123 significantly augmented forearm blood flow: by 54% in control patients ($p < 0.001$) and by 30% in CHF patients ($p < 0.002$) from baseline to 90 min of infusion. In contrast, selective ET_B receptor blockade via BQ-788 infusion resulted in progressive vasoconstriction, with reductions in forearm blood flow of 15% in controls ($p = 0.04$ *vs.* baseline) and 9% in CHF patients ($p = 0.0006$ *vs.* baseline) (90).

Similar findings were reported in a study by Goddard *et al.* of ET_A and ET_B receptor blockade in 8 patients with hypertension in the setting of chronic renal failure. ET_A receptor antagonism was associated with vasodilator effects, with substantial declines in blood pressure secondary to renal vasodilation. Conversely, combined ET_A/ET_B receptor blockade was less effective in lowering blood pressure, exerted no beneficial effects on renal hemodynamics and was associated with reduced ET-1 clearance. ET_B receptor blockade alone elicited marked systemic and renal vasoconstriction (91).

Selective ET_A receptor antagonism was also associated with vasodilator effects in patients with another form of endothelial dysfunction: atherosclerosis. In a Doppler ultrasonographic study of 44 patients with coronary heart disease (CHD) and/or atherosclerotic risk factors, infusion of a selective ET_A receptor antagonist (BQ-123) exerted systemic, epicardial and pulmonary vasodilating effects, lowering arterial blood pressure by 8 mmHg (6%; $p < 0.001$) and mean PAP by 1.9 mmHg (15%; $p < 0.01$) from baseline (92). ET_A blockade also increased epicardial artery minimum luminal diameter by approximately 6% ($p < 0.001$) and caused progressive microvascular dilation, with an increase in coronary blood flow of 9.1% ($p < 0.02$) and a decrease in coronary vascular resistance of 12% ($p < 0.02$) compared with baseline.

Selective ET_A receptor blockade also reversed epicardial vasoconstriction in response to cold pressor testing, particularly in arterial segments with marked endothelial dysfunction. An arteriovenous difference in ET-1 levels developed following BQ-123 infusion, indicating enhanced cardiac clearance of this peptide through the (unblocked) interaction of ET-1 with ET_B receptors during ET_A receptor blockade (92).

Sitaxsentan

Pharmacological actions

Sitaxsentan (Fig. 1) is a potent ($K_i = 0.43$ nM) and highly selective ET_A receptor antagonist, with 6,500-fold higher selectivity for the ET_A compared with the ET_B receptor (93). This compound inhibits ET-1-induced stimulation of phosphoinositide turnover with a K_i of 0.686 nM and a pA_2 of 8.0 (93). Sitaxsentan has a terminal elimination half-life of 10 h in humans, and steady state is achieved within approximately 6 days.

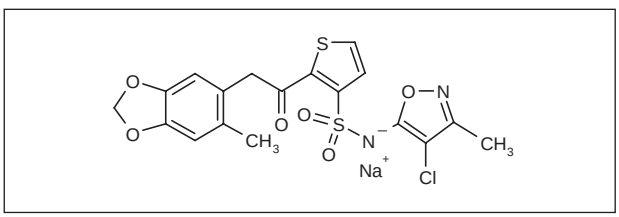

Fig. 1. Molecular structure of sitaxsentan.

Treatment with sitaxsentan both prevented and attenuated ongoing experimental PAH in rats exposed to hypoxia (acute or chronic) or monocrotaline, reversing vasoconstriction and both pulmonary vascular and right ventricular remodeling. Upon acute exposure of rats to normobaric hypoxia (10% O_2 at 1 atm) for 90 min, there is a biphasic increase in mean PAP, with peak levels of approximately 25 mmHg, followed by a return to baseline following exposure to room air. Infusion of sitaxsentan 5 mg/kg 10 min before the onset of hypoxia prevented hypoxia-induced vasoconstriction, with mean PAP values similar to those for rats exposed to air. However, administration of a selective ET_B receptor antagonist (BQ-788) did not affect pulmonary vasoconstriction, but did attenuate the biphasic pressure response to hypoxia (94).

Sitaxsentan also prevented and reversed PAH associated with chronic hypoxic exposure. Whereas 2 weeks of hypoxic exposure significantly raised PAP (to 34 mmHg) compared with control rats (peak = 17 mmHg; $p < 0.0001$), oral administration of sitaxsentan starting 2 days prior to hypoxic exposure and continuing during hypoxia significantly blunted this increase (peak = 22 mmHg; $p < 0.0001$ *vs.* untreated hypoxia group). When administered during 4 weeks of ongoing hypoxic exposure, sitaxsentan also dose-dependently reversed the increase in mean PAP, to 26 mmHg (15 mg/kg/day) and 21 mmHg (30 mg/kg/day) as compared to 37 mmHg for rats exposed to 6 weeks of hypoxia without sitaxsentan ($p < 0.0001$ for each comparison *vs.* untreated hypoxic group). Sitaxsentan did not affect mean systemic arterial pressure or heart rate (94).

Together with rises in mean PAP, rats exposed to chronic hypoxia for 2-6 weeks also exhibit right ventricular hypertrophy, with increases in right ventricular weights to more than two times the weights of hearts from rats exposed to room air. When administered during the first 2 of 6 weeks of hypoxia, sitaxsentan significantly reduced (by approximately 50%) right ventricular enlargement compared with untreated hypoxic rats ($p < 0.0001$) and also attenuated (by 30-37%) established right ventricular hypertrophy when administered during the final 4 weeks of hypoxia. Ratios of right ventricle and right atrium to left ventricle plus septum were reduced by 46% and 85%, respectively, compared with the normalized values in hypoxic controls (94).

Sitaxsentan also prevented and attenuated hypoxia-induced pulmonary vascular remodeling. Whereas percent medial wall thickness within the pulmonary artery rose from 14.7% in rats exposed to room air to 24.9% in rats following 2 weeks of hypoxic exposure, wall thickness increased to only 17.2% in animals treated with sitaxsentan 15 mg/kg for 2 weeks ($p < 0.0001$ *vs.* untreated hypoxic rats). These findings are summarized in Figure 2 (94).

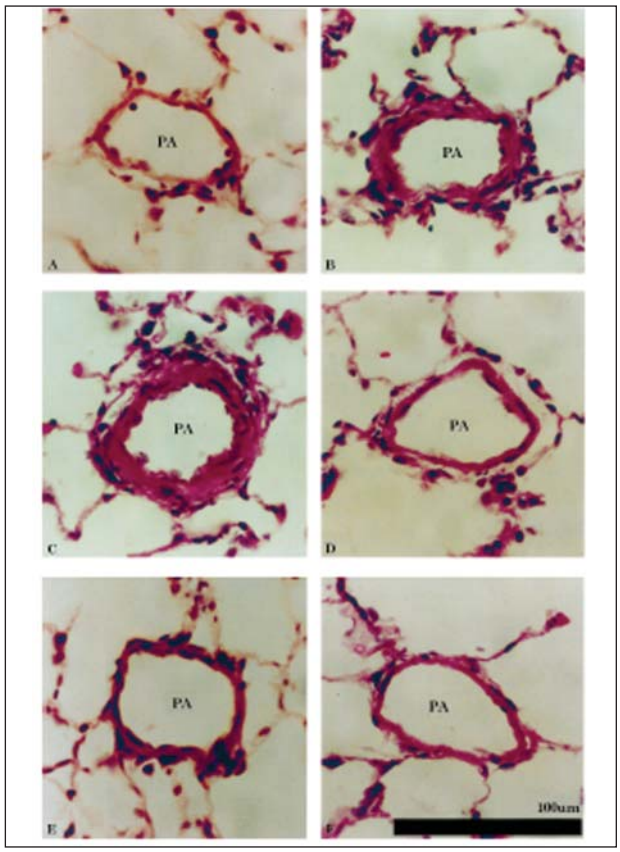

Fig. 2. Representative light micrographs of small pulmonary arteries (PA; 50-100 μm) from rats exposed to chronic hypoxia (10% O_2 for 2 or 6 weeks) or room air in the presence or absence of sitaxsentan. A: air control; B: 2 weeks 10% O_2; C: 6 weeks 10% O_2; D: 2 weeks 10% O_2 + 2 weeks sitaxsentan 15 mg/kg/day; E: 6 weeks 10% O_2 + 4 weeks sitaxsentan 15 mg/kg/day; F: 6 weeks 10% O_2 + 4 weeks sitaxsentan 30 mg/kg/day. Magnification: 400X; bar represents 100 μm. Reproduced with permission from Ref. 94 and Elsevier.

Consistent with the vasoconstriction and vascular and right ventricular remodeling, there was a 4-fold rise in plasma ET-1 levels from baseline following 2 weeks of hypoxia (from 5.0 to 21.4 pg/ml) and a > 5-fold increase (to 27.9 pg/ml) from baseline following 6 weeks of hypoxia. When administered during the first 2 weeks of hypoxic exposure, sitaxsentan significantly prevented the increase in endogenous ET-1, resulting in a level of 9.8 pg/ml ($p < 0.01$ *vs.* hypoxic rats) (94). This effect may be attributed to selective ET_A receptor antagonism by sitaxsentan, which permits ET_B receptor-mediated ET-1 clearance.

Similar findings were reported in rats exposed to 40 mg/kg monocrotaline, a pyrrolizidine alkaloid that elicits pulmonary vascular damage in a wide range of animal models following a single s.c. injection. Administration of sitaxsentan 50 mg/kg prevented monocrotaline-induced rises in right ventricular weight and right ventricular systolic pressure. Whereas right ventricular weight rose 78% in monocrotaline-treated rats compared with controls (340 mg *vs.* 191 mg; $p < 0.0001$), sitaxsentan dose-dependently lowered right ventricular weight, with a significant difference in rats treated with monocrotaline and sitaxsentan 50 mg/kg as compared with rats exposed to monocrotaline alone ($p < 0.0001$) (94).

Sitaxsentan also prevented pulmonary vascular remodeling associated with monocrotaline exposure. Compared with controls, rats exposed to monocrotaline had an approximately 78% increase in pulmonary artery percent wall thickness (from 9.1% to 16.1%) 3 weeks after monocrotaline administration, whereas rats treated with sitaxsentan 50 mg/kg together with monocrotaline had a pulmonary artery percent wall thickness of 10.9% ($p < 0.007$ *vs.* monocrotaline-treated rats) (94).

Clinical studies

The first published clinical experience with sitaxsentan dates to 2002, when Barst and colleagues reported the results of an open-label trial evaluating sitaxsentan in 6 children and 14 adults with functional class II-IV idio-

pathic PAH (n=8; 40%) or PAH associated with either collagen vascular disease (n=2; 10%) or congenital systemic-to-pulmonary shunts (n=10; 50%) (95). The mean patient age was 36 years, and most patients (70%) were female. Patients with portal hypertension or chronic liver disease, uncontrolled systemic hypertension, and/or who were receiving treatment with either calcium channel blockers or epoprostenol were excluded. Sitaxsentan therapy (100-500 mg b.i.d.) for 12 weeks significantly enhanced exercise capacity and cardiopulmonary hemodynamics compared with baseline. The 6MW distance rose from a mean of 466 m to 504 m at week 6, a 37-m improvement (p = 0.012 *vs.* baseline). The increase in 6MW distance rose to 48 m at week 12 (p = 0.006) when missing data from patients discontinuing treatment were imputed. Change in 6MW distance from baseline to week 12 correlated with changes in mixed venous oxygen saturation (r =0.56; p = 0.029) (95).

Treatment with sitaxsentan for 12 weeks also significantly improved cardiopulmonary hemodynamics, lowering mean PAP by 17.5%, from 63 mmHg at baseline to 52 mmHg (p = 0.0002), and PVR index by 30%, from 20 U/m^2 to 14 U/m^2 (p = 0.008) In contrast, sitaxsentan elicited no significant hemodynamic effects within 6 h of administration. This suggests that the ET_A receptor antagonist functions chiefly by reversing vasoconstriction and pulmonary vascular remodeling over a period of weeks rather than via short-term vasodilating effects (95).

The most frequent adverse events were attributable to vasodilation in nonpulmonary vascular beds, including nasal congestion, flushing and headache. Other adverse events included elevated prothrombin time (PT)/International Normalized Ratio (INR), edema and nausea. The elevations in PT/INR were probably attributable to a pharmacokinetic interaction with warfarin, because sitaxsentan is a potent inhibitor of cytochrome P-450 (CYP2C9), the major enzyme involved in the metabolism of (*S*)-warfarin. In 3 patients, elevated PT/INR was serious. Other serious adverse events occurring in 1 patient each included systemic hypotension, pulmonary edema, syncope, bronchitis, pneumonia, chest pain and anemia. Both mean hemoglobin (–11%) and hematocrit (–4%) values decreased significantly with long-term sitaxsentan treatment, but no bleeding episodes were reported (95). A total of 7 patients experienced mild asymptomatic elevations in serum transaminase values. Although transient in 6 patients, 1 patient subsequently developed concomitant abnormalities in alkaline phosphatase and bilirubin, with acute hepatitis in an extension phase. The patient's liver function normalized after discontinuation of sitaxsentan. One patient had marked increases in liver function enzyme values and developed severe hepatitis in the extension phase that was fatal (95). Abnormalities in liver function tests are recognized class effects of ETRAs (95-97), and serum transaminase monitoring is warranted during treatment with an ETRA (95).

Sitaxsentan was subsequently evaluated at lower doses (100 and 300 mg once daily) in the Sitaxsentan To Relieve Impaired Exercise (STRIDE-1) trial (98). A total of 178 patients with idiopathic PAH (53%) or PAH related to either congenital heart disease (24%) or connective tissue disease (24%) were randomized to daily treatment with sitaxsentan 100 (n=55) or 300 mg (n=63), or placebo (n=60), for 12 weeks. Approximately two-thirds of the patients were functional class III, with the remainder being classes II (33%) and IV (1%). Mean 6MW distance at baseline was 398 m, mean PAP was 54 mmHg and mean PVR was 958 dyn/s/cm^5 (98).

Sitaxsentan treatment significantly enhanced exercise capacity, cardiopulmonary hemodynamics and functional class compared with placebo. As shown in Figure 3, 6MW distance increased by 22 m in patients randomized to sitaxsentan 100 mg and by 20 m in those randomized to sitaxsentan 300 mg, in contrast to a decline of 13 m in the placebo group; thus, the treatment effects were an increase of 35 m (p < 0.01) for the low-dose sitaxsentan group and 33 m (p < 0.01) for the high-dose sitaxsentan group (98). Functional class improved in 16 of 55 (29%) patients receiving sitaxsentan 100 mg and 19 of 63 (30%) of those receiving sitaxsentan 300 mg (p < 0.02 for both sitaxsentan treatment groups *vs.* placebo). None of the patients receiving 100 mg and only 1 of the patients receiving 300 mg had a worsening in functional class compared with 3 patients (5%) in the placebo group. Clinical worsening, defined as death, epoprostenol use, transplantation or atrial septostomy, occurred in only 4 patients, 3 of whom were in the placebo group (7% of group). No patient randomized to sitaxsentan 100 mg experienced clinical worsening. Mean right atrial pressure was also significantly decreased compared with placebo (p < 0.005). Decreases in PAP were observed in both the 100-mg (p = NS) and 300-mg groups (p < 0.001) compared with placebo (98). At both doses, sitaxsentan treatment also significantly enhanced cardiopulmonary hemodynamics. For instance, treatment with sitaxsentan 100 mg for 12 weeks increased cardiac index by approximately 12% (p < 0.02 *vs.* placebo) and lowered PVR by approximately 21% (p < 0.001) (98).

Sitaxsentan was well tolerated. As in the pilot study, the chief adverse events were headache, which occurred in 45% of sitaxsentan patients compared with 34% of placebo controls, peripheral edema (21% *vs.* 17%), nausea (20% *vs.* 19%), nasal congestion (18% *vs.* 10%) and dizziness (12% *vs.* 10%). Elevations in PT or INR occurred in 14% of patients randomized to sitaxsentan 100 mg compared with 5% of placebo controls. The majority of patients (about 80%) in both of these treatment groups received warfarin therapy (98). Notably, no patient treated with sitaxsentan 100 mg had > 3-fold elevations in aminotransferase values compared with 3% for placebo; 10% of patients in the high-dose group experienced > 3-fold elevations in aminotransferase values. All abnormalities in liver function tests were reversible and asymptomatic (98).

STRIDE-1 also contained a pharmacokinetic aspect, which demonstrated nonlinearity in sitaxsentan elimination at the 300-mg dose: systemic exposure (AUC) at

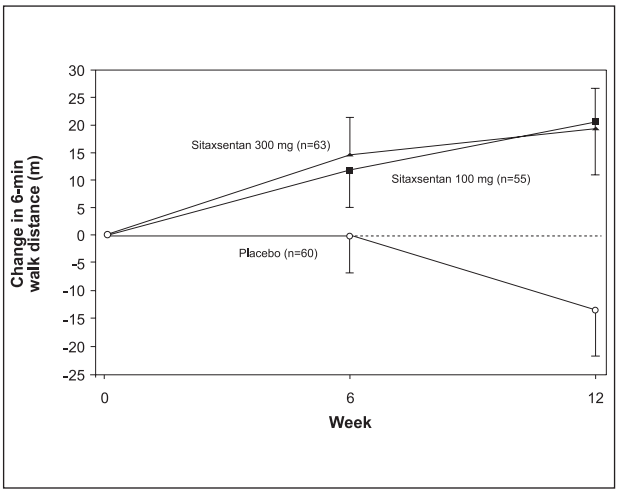

Fig. 3. Mean (± SE) change in 6-min walk distance from baseline to week 12 in placebo and sitaxsentan groups. Pairwise (*vs.* placebo) *p* values are from analysis of covariance including baseline response in the model and are adjusted for multiple comparisons using Dunnett's method. *p* values < 0.01 for the comparison between each sitaxsentan dose (100 and 300 mg) and placebo. Reproduced with permission from Ref. 98 and the American Thoracic Society.

steady state was approximately 11-fold higher in the high-dose sitaxsentan group compared with the low-dose sitaxsentan group (98). In further STRIDE investigations, including STRIDE-2 (which contains a bosentan treatment arm) and STRIDE-6 (which evaluates effects of sitaxsentan in bosentan failures), only once-daily 50- and 100-mg doses of sitaxsentan were evaluated.

Unlike prior studies involving ETRAs (97), STRIDE-1 did not exclude patients with mild disease. In particular, no upper limit was placed on 6MW distance, whereas other studies excluded patients with baseline 6MW distances > 450 m, and patients in functional class II were eligible, whereas other studies excluded these patients. As a result, there was a ceiling effect with regard to the impact of sitaxsentan in patients in STRIDE-1, who had overall milder disease than patients in prior studies (98).

When examining the subset of the STRIDE-1 population who would have qualified by the more stringent "traditional criteria" (*i.e.*, patients with more severe disease, *e.g.*, functional class III and IV, and excluding CHD patients or those who walked > 450 m at baseline) used in other PAH trials, clinical improvements with sitaxsentan were more pronounced. Mean 6MW distance increased by 65 m (20.9%) relative to placebo. Improvements in hemodynamics were also more pronounced, with mean PAP declining by approximately 10% compared with placebo ($p = 0.03$). Sitaxsentan therapy also significantly lowered PVR by approximately 34% ($p < 0.0001$) and increased cardiac index by approximately 22% ($p < 0.0001$). A total of 45% of patients in the "traditional criteria" subset who were treated with sitaxsentan demonstrated improvement in functional class, with no patient worsening, in contrast to 9% of placebo controls who had improvement and 9% who showed worsening ($p = 0.0005$) (99).

A Canadian open-label extension of STRIDE-1 showed that clinical benefits were sustained for up to 1 year (100). Among 11 patients, 1 experienced clinical worsening at 7 months, requiring the institution of continuous i.v. epoprostenol therapy. It is important to recognize that patients with rapid clinical deterioration are not candidates for sitaxsentan or other ETRAs and should be considered for therapy with a prostacyclin analogue. Among the remaining 10 patients, mean 6MW distance increased by 50 m (13%; to 436 m) from baseline to 1 year ($p = 0.04$). Whereas 9 of 10 patients were in functional class III at baseline, all 10 were in functional class II after 1 year of sitaxsentan treatment ($p < 0.01$). Cardiac output increased by approximately 25% ($p = 0.009$) and PVR fell by 21% ($p = 0.04$) from baseline to 1 year of sitaxsentan treatment (100). Based on a presentation at the 2005 American Thoracic Society (ATS) in San Diego, California, clinical benefits were sustained at 2 years of treatment, with all eligible patients remaining in functional class II and the 6MW distance holding at 440 m ($p = 0.02$ *vs.* baseline) (101).

Sitaxsentan was well tolerated, with no serious adverse events observed. Headache, peripheral edema, nasal congestion and nausea were the chief adverse events. No patient had abnormal liver function tests or complications related to the interaction between sitaxsentan and warfarin (100). No evidence of hepatotoxicity was observed in these patients for up to 2 years (101).

Although bosentan represented a major clinical advance in the treatment of PAH, up to 11% of patients experience abnormal liver function tests, with some discontinuing therapy (102). Furthermore, on the basis of findings from the Bosentan Randomized Trial of Endothelin Antagonist Therapy (BREATHE-1) trial, approximately 60% of patients did not improve in functional class following 16 weeks of therapy with the ETRA at doses of 62.5-125 mg twice daily (97). Sitaxsentan may be a viable treatment option for patients with suboptimal responses to bosentan, either because of abnormal liver function test results or insufficient efficacy outcomes (103-105).

The recently completed STRIDE-2 study compared sitaxsentan to placebo double-blind and also included a single-blind bosentan arm for comparison. The primary endpoint was 6MW distance. The placebo-subtracted treatment effect was 31.4 m for sitaxsentan 100 mg ($p = 0.03$), 24.2 m (p = NS) for sitaxsentan 50 mg and 29.5 m ($p = 0.05$) for bosentan. Sitaxsentan 100 mg also demonstrated significant improvements in WHO functional class ($p = 0.04$). Abnormal liver function tests occurred in 3.2% of patients on sitaxsentan 100 mg compared with 4.9% for sitaxsentan 50 mg, 6.5% for placebo and 11.5% for bosentan (106).

Conclusions

Pulmonary arterial hypertension is a rare but frequently fatal condition marked by progressive vasocon-

striction and pulmonary vascular remodeling. Increased synthesis of ET-1 has been identified as central to the pathology of PAH. Given that ET_A receptors appear to be central to the vasoconstriction, intimal proliferation, vessel wall thickening and fibrosis associated with PAH, whereas ET_B receptors mediate vasodilator synthesis and ET-1 clearance, selective ET_A receptor blockade may be desirable. However, the issue of whether selective or nonselective ETRAs are more effective therapies for PAH can be adjudicated only via active comparator trials and other forms of clinical experience. As a highly ET_A receptor-selective agent, sitaxsentan is a promising treatment option, significantly enhancing exercise capacity, functional capacity and cardiopulmonary hemodynamics in PAH patients.

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References

1. Rich, S., McLaughlin, V.V. *Pulmonary hypertension*. In: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. D.P. Zipes, P. Libby, R.O. Bonow, E. Braunwald (Eds.). Elsevier Saunders, Philadelphia, 2005, 1807-42.

2. Rich, S., Dantzker, D.R., Ayres, S.M. et al. *Primary pulmonary hypertension. A national prospective study*. Ann Intern Med 1987, 107: 216-23.

3. D'Alonzo, G.E., Barst, R.J., Ayres, S.M. et al. *Survival in patients with primary pulmonary hypertension. Results from a national prospective registry*. Ann Intern Med 1991, 115: 343-9.

4. Rubin, L.J. *Diagnosis and management of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines*. Chest 2004, 126: 7S-10S.

5. Abenhaim, L., Moride, Y., Brenot, F. et al., for the International Primary Pulmonary Hypertension Study Group. *Appetite-suppressant drugs and the risk of primary pulmonary hypertension*. New Engl J Med 1996, 335: 609-16.

6. Morse, J.H., Jones, A.C., Barst, R.J., Hodge, S.E., Wilhelmsen, K.C., Nygaard, T.G. *Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31-q32*. Circulation 1997, 95: 2603-6.

7. Nichols, W.C., Koller, D.L., Slovis, B. et al. *Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31-32*. Nat Genet 1997, 15: 277-80.

8. The International PPH Consortium, Lane, K.B., Machado, R.D., Pauciulo, M.W. et al. *Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension*. Nat Genet 2000, 26: 81-4.

9. Deng, Z., Morse, J.H., Slager, S.L. et al. *Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene*. Am J Hum Genet 2000, 67: 737-44.

10. Newman, J.H., Wheeler, L., Lane, K.B. et al. *Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred*. New Engl J Med 2001, 345: 319-24.

11. Massague, J., Blain, S.W., Lo, R.S. *TGFbeta signaling in growth control, cancer, and heritable disorders*. Cell 2000, 103: 295-309.

12. Du, L., Sullivan, C.C., Chu, D. et al. *Signaling molecules in nonfamilial pulmonary hypertension*. New Engl J Med 2003, 348: 500-9.

13. Kawut, S.M., Taichman, D.B., Archer-Chicko, C.L., Palevsky, H.I., Kimmel, S.E. *Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis*. Chest 2003, 123: 344-50.

14. Gladwin, M.T., Sachdev, V., Jison, M.L. et al. *Pulmonary hypertension as a risk factor for death in patients with sickle cell disease*. New Engl J Med 2004, 350: 886-95.

15. Farber, H.W., Loscalzo, J. *Pulmonary arterial hypertension*. New Engl J Med 2004, 351: 1655-65.

16. Humbert, M., Morrell, N.W., Archer, S.L. et al. *Cellular and molecular pathobiology of pulmonary arterial hypertension*. J Am Coll Cardiol 2004, 43: 13S-24S.

17. Giaid, A., Saleh, D. *Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension*. New Engl J Med 1995, 333: 214-21.

18. Kaneko, F.T., Arroliga, A.C., Dweik, R.A. et al. *Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension*. Am J Respir Crit Care Med 1998, 158: 917-23.

19. Christman, B.W., McPherson, C.D., Newman, J.H. et al. *An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension*. New Engl J Med 1992, 327: 70-5.

20. Tuder, R.M., Cool, C.D., Geraci, M.W. et al. *Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension*. Am J Respir Crit Care Med 1999, 159: 1925-32.

21. Petkov, V., Mosgoeller, W., Ziesche, R. et al. *Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension*. J Clin Invest 2003, 111: 1339-46.

22. MacLean, M.R., Herve, P., Eddahibi, S., Adnot, S. *5-Hydroxytryptamine and the pulmonary circulation: Receptors, transporters and relevance to pulmonary arterial hypertension*. Br J Pharmacol 2000, 131: 161-8.

23. Herve, P., Launay, J.M., Scrobohaci, M.L. et al. *Increased plasma serotonin in primary pulmonary hypertension*. Am J Med 1995, 99: 249-54.

24. Morecroft, I., Heeley, R.P., Prentice, H.M., Kirk, A., MacLean, M.R. *5-Hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: Importance of the 5-HT1B receptor*. Br J Pharmacol 1999, 128: 730-4.

25. Keegan, A., Morecroft, I., Smillie, D., Hicks, M.N., MacLean, M.R. *Contribution of the 5-HT(1B) receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT(1B)-receptor knockout mice and the 5-HT(1B/1D)-receptor antagonist GR127935*. Circ Res 2001, 89: 1231-9.

26. Eddahibi, S., Humbert, M., Fadel, E. et al. *Serotonin transporter overexpression is responsible for pulmonary artery*

smooth muscle hyperplasia in primary pulmonary hypertension. J Clin Invest 2001, 108: 1141-50.

27. Marcos, E., Adnot, S., Pham, M.H. et al. *Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension*. Am J Respir Crit Care Med 2003, 168: 487-93.

28. Gerber, J.G., Voelkel, N., Nies, A.S., McMurtry, I.F., Reeves, J.T. *Moderation of hypoxic vasoconstriction by infused arachidonic acid: Role of PGI2*. J Appl Physiol 1980, 49: 107-12.

29. Yang, X., Long, L., Southwood, M. et al. *Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension*. Circ Res 2005, 96: 1053-63.

30. Yuan, X.J., Wang, J., Juhaszova, M., Gaine, S.P., Rubin, L.J. *Attenuated K+ channel gene transcription in primary pulmonary hypertension*. Lancet 1998, 351: 726-7.

31. Michelakis, E.D., McMurtry, M.S., Wu, X.C. et al. *Dichloroacetate, a metabolic modulator, prevents and reverses chronic hypoxic pulmonary hypertension in rats: Role of increased expression and activity of voltage-gated potassium channels*. Circulation 2002, 105: 244-50.

32. Geraci, M.W., Moore, M., Gesell, T. et al. *Gene expression patterns in the lungs of patients with primary pulmonary hypertension: A gene microarray analysis*. Circ Res 2001, 88: 555-62.

33. Guignabert, C., Raffestin, B., Benferhat, R. et al. *Serotonin transporter inhibition prevents and reverses monocrotaline-induced pulmonary hypertension in rats*. Circulation 2005, 111: 2812-9.

34. Hoeper, M.M. *Pulmonary hypertension in collagen vascular disease*. Eur Respir J 2002, 19: 571-6.

35. Jamison, B.M., Michel, R.P. *Different distribution of plexiform lesions in primary and secondary pulmonary hypertension*. Hum Pathol 1995, 26: 987-93.

36. Ivy, D.D., McMurtry, I.F., Colvin, K. et al. *Development of occlusive neointimal lesions in distal pulmonary arteries of endothelin B receptor-deficient rats: A new model of severe pulmonary arterial hypertension*. Circulation 2005, 111: 2988-96.

37. Tuder, R.M., Groves, B., Badesch, D.B., Voelkel, N.F. *Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hypertension*. Am J Pathol 1994, 144: 275-85.

38. Tuder, R.M., Chacon, M., Alger, L. et al. *Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: Evidence for a process of disordered angiogenesis*. J Pathol 2001, 195: 367-74.

39. Benisty, J.I., McLaughlin, V.V., Landzberg, M.J. et al. *Elevated basic fibroblast growth factor levels in patients with pulmonary arterial hypertension*. Chest 2004, 126: 1255-61.

40. Barst, R.J., McGoon, M., Torbicki, A. et al. *Diagnosis and differential assessment of pulmonary arterial hypertension*. J Am Coll Cardiol 2004, 43: 40S-7S.

41. Borgeson, D.D., Seward, J.B., Miller, F.A., Jr., Oh, J.K., Tajik, A.J. *Frequency of Doppler measurable pulmonary artery pressures*. J Am Soc Echocardiogr 1996, 9: 832-7.

42. Badesch, D.B., Abman, S.H., Ahearn, G.S. et al. *Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines*. Chest 2004, 126: 35S-62S.

43. Galié, N., Ghofrani, H.A., Torbicki, A. et al., for the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) Study Group. *Sildenafil citrate therapy for pulmonary arterial hypertension*. New Engl J Med 2005, 353: 2148-57.

44. Hoeper, M.M., Faulenbach, C., Golpon, H., Winkler, J., Welte, T., Niedermeyer, J. *Combination therapy with bosentan and sildenafil in idiopathic pulmonary arterial hypertension*. Eur Respir J 2004, 24: 1007-10.

45. Humbert, M., Barst, R.J., Robbins, I.M. et al. *Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2*. Eur Respir J 2004, 24: 353-9.

46. Luscher, T.F., Barton, M. *Endothelins and endothelin receptor antagonists: Therapeutic considerations for a novel class of cardiovascular drugs*. Circulation 2000, 102: 2434-40.

47. Hickey, K.A., Rubanyi, G., Paul, R.J., Highsmith, R.F. *Characterization of a coronary vasoconstrictor produced by cultured endothelial cells*. Am J Physiol 1985, 248: C550-6.

48. Gillespie, M.N., Owasoyo, J.O., McMurtry, I.F., O'Brien, R.F. *Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture*. J Pharmacol Exp Ther 1986, 236: 339-43.

49. O'Brien, R.F., Robbins, R.J., McMurtry, I.F. *Endothelial cells in culture produce a vasoconstrictor substance*. J Cell Physiol 1987, 132: 263-70.

50. Yanagisawa, M., Kurihara, H., Kimura, S. et al. *A novel potent vasoconstrictor peptide produced by vascular endothelial cells*. Nature 1988, 332: 411-5.

51. Yanagisawa, M., Inoue, A., Ishikawa, T. et al. *Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide*. Proc Natl Acad Sci USA 1988, 85: 6964-7.

52. Masaki, T. *The discovery of endothelins*. Cardiovasc Res 1998, 39: 530-3.

53. Gu, X.H., Liu, J.J., Dillon, J.S., Nayler, W.G. *The failure of endothelin to displace bound, radioactively-labelled, calcium antagonists (PN 200/110, D888 and diltiazem)*. Br J Pharmacol 1989, 96: 262-4.

54. Resink, T.J., Scott-Burden, T., Buhler, F.R. *Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells*. Biochem Biophys Res Commun 1988, 157: 1360-8.

55. Simonson, M.S., Wann, S., Mene, P. et al. *Endothelin stimulates phospholipase C, Na+/H+ exchange, c-fos expression, and mitogenesis in rat mesangial cells*. J Clin Invest 1989, 83: 708-12.

56. Sugiura, M., Inagami, T., Hare, G.M., Johns, J.A. *Endothelin action: Inhibition by a protein kinase C inhibitor and involvement of phosphoinositols*. Biochem Biophys Res Commun 1989, 158: 170-6.

57. Benigni, A., Remuzzi, G. *Endothelin antagonists*. Lancet 1999, 353: 133-8.

58. Ovadia, B., Reinhartz, O., Fitzgerald, R. et al. *Alterations in ET-1, not nitric oxide, in 1-week-old lambs with increased pulmonary blood flow*. Am J Physiol Heart Circ Physiol 2003, 284: H480-90.

59. Dupuis, J., Cernacek, P., Tardif, J.C. et al. *Reduced pulmonary clearance of endothelin-1 in pulmonary hypertension*. Am Heart J 1998, 135: 614-20.

60. Stewart, D.J., Levy, R.D., Cernacek, P., Langleben, D. *Increased plasma endothelin-1 in pulmonary hypertension: Marker or mediator of disease?* Ann Intern Med 1991, 114: 464-9.

61. Giaid, A., Yanagisawa, M., Langleben, D. et al. *Expression of endothelin-1 in the lungs of patients with pulmonary hypertension*. New Engl J Med 1993, 328: 1732-9.

62. Rubens, C., Ewert, R., Halank, M. et al. *Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension*. Chest 2001, 120: 1562-9.

63. Dupuis, J., Stewart, D.J., Cernacek, P., Gosselin, G. *Human pulmonary circulation is an important site for both clearance and production of endothelin-1*. Circulation 1996, 94: 1578-84.

64. Davie, N., Haleen, S.J., Upton, P.D. et al. *ET(A) and ET(B) receptors modulate the proliferation of human pulmonary artery smooth muscle cells*. Am J Respir Crit Care Med 2002, 165: 398-405.

65. Alberts, G.F., Peifley, K.A., Johns, A., Kleha, J.F., Winkles, J.A. *Constitutive endothelin-1 overexpression promotes smooth muscle cell proliferation via an external autocrine loop*. J Biol Chem 1994, 269: 10112-8.

66. Ohlstein, E.H., Arleth, A., Bryan, H., Elliott, J.D., Sung, C.P. *The selective endothelin ETA receptor antagonist BQ123 antagonizes endothelin-1-mediated mitogenesis*. Eur J Pharmacol 1992, 225: 347-50.

67. Panettieri, R.A., Jr., Goldie, R.G., Rigby, P.J., Eszterhas, A.J., Hay, D.W. *Endothelin-1-induced potentiation of human airway smooth muscle proliferation: An ETA receptor-mediated phenomenon*. Br J Pharmacol 1996, 118: 191-7.

68. Hirata, Y., Takagi, Y., Fukuda, Y., Marumo, F. *Endothelin is a potent mitogen for rat vascular smooth muscle cells*. Atherosclerosis 1989, 78: 225-8.

69. Komuro, I., Kurihara, H., Sugiyama, T., Yoshizumi, M., Takaku, F., Yazaki, Y. *Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells*. FEBS Lett 1988, 238: 249-52.

70. Janakidevi, K., Fisher, M.A., Del Vecchio, P.J., Tiruppathi, C., Figge, J., Malik, A.B. *Endothelin-1 stimulates DNA synthesis and proliferation of pulmonary artery smooth muscle cells*. Am J Physiol 1992, 263: C1295-301.

71. Yang, Z., Krasnici, N., Luscher, T.F. *Endothelin-1 potentiates human smooth muscle cell growth to PDGF: Effects of ETA and ETB receptor blockade*. Circulation 1999, 100: 5-8.

72. Weissberg, P.L., Witchell, C., Davenport, A.P., Hesketh, T.R., Metcalfe, J.C. *The endothelin peptides ET-1, ET-2, ET-3 and sarafotoxin S6b are co-mitogenic with platelet-derived growth factor for vascular smooth muscle cells*. Atherosclerosis 1990, 85: 257-62.

73. Dawes, K.E., Cambrey, A.D., Campa, J.S. et al. *Changes in collagen metabolism in response to endothelin-1: Evidence for fibroblast heterogeneity*. Int J Biochem Cell Biol 1996, 28: 229-38.

74. Rizvi, M.A., Katwa, L., Spadone, D.P., Myers, P.R. *The effects of endothelin-1 on collagen type I and type III synthesis in cultured porcine coronary artery vascular smooth muscle cells*. J Mol Cell Cardiol 1996, 28: 243-52.

75. Ammarguellat, F.Z., Gannon, P.O., Amiri, F., Schiffrin, E.L. *Fibrosis, matrix metalloproteinases, and inflammation in the heart of DOCA-salt hypertensive rats: Role of ET(A) receptors*. Hypertension 2002, 39: 679-84.

76. Marini, M., Carpi, S., Bellini, A., Patalano, F., Mattoli, S. *Endothelin-1 induces increased fibronectin expression in human bronchial epithelial cells*. Biochem Biophys Res Commun 1996, 220: 896-9.

77. Shi-Wen, X., Chen, Y., Denton, C.P. et al. *Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts*. Mol Biol Cell 2004, 15: 2707-19.

78. Guidry, C., Hook, M. *Endothelins produced by endothelial cells promote collagen gel contraction by fibroblasts*. J Cell Biol 1991, 115: 873-80.

79. Dupuis, J., Goresky, C.A., Fournier, A. *Pulmonary clearance of circulating endothelin-1 in dogs in vivo: Exclusive role of ETB receptors*. J Appl Physiol 1996, 81: 1510-5.

80. Fukuroda, T., Fujikawa, T., Ozaki, S., Ishikawa, K., Yano, M., Nishikibe, M. *Clearance of circulating endothelin-1 by ETB receptors in rats*. Biochem Biophys Res Commun 1994, 199: 1461-5.

81. Bohm, F., Pernow, J., Lindstrom, J., Ahlborg, G. *ETA receptors mediate vasoconstriction, whereas ETB receptors clear endothelin-1 in the splanchnic and renal circulation of healthy men*. Clin Sci (Lond) 2003, 104: 143-51.

82. Pollock, D.M., Keith, T.L., Highsmith, R.F. *Endothelin receptors and calcium signaling*. FASEB J 1995, 9: 1196-204.

83. Ozaki, S., Ohwaki, K., Ihara, M., Fukuroda, T., Ishikawa, K., Yano, M. *ETB-mediated regulation of extracellular levels of endothelin-1 in cultured human endothelial cells*. Biochem Biophys Res Commun 1995, 209: 483-9.

84. Naomi, S., Iwaoka, T., Disashi, T. et al. *Endothelin-1 inhibits endothelin-converting enzyme-1 expression in cultured rat pulmonary endothelial cells*. Circulation 1998, 97: 234-6.

85. Murakoshi, N., Miyauchi, T., Kakinuma, Y. et al. *Vascular endothelin-B receptor system in vivo plays a favorable inhibitory role in vascular remodeling after injury revealed by endothelin-B receptor-knockout mice*. Circulation 2002, 106: 1991-8.

86. Ohuchi, T., Kuwaki, T., Ling, G.Y. et al. *Elevation of blood pressure by genetic and pharmacological disruption of the ETB receptor in mice*. Am J Physiol 1999, 276: R1071-7.

87. Ivy, D.D., McMurtry, I.F., Yanagisawa, M. et al. *Endothelin B receptor deficiency potentiates ET-1 and hypoxic pulmonary vasoconstriction*. Am J Physiol Lung Cell Mol Physiol 2001, 280: L1040-8.

88. Carpenter, T., Schomberg, S., Steudel, W. et al. *Endothelin B receptor deficiency predisposes to pulmonary edema formation via increased lung vascular endothelial cell growth factor expression*. Circ Res 2003, 93: 456-63.

89. Verhaar, M.C., Strachan, F.E., Newby, D.E. et al. *Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade*. Circulation 1998, 97: 752-6.

90. Love, M.P., Ferro, C.J., Haynes, W.G. et al. *Endothelin receptor antagonism in patients with chronic heart failure*. Cardiovasc Res 2000, 47: 166-72.

91. Goddard, J., Johnston, N.R., Hand, M.F. et al. *Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: A comparison of selective and combined endothelin receptor blockade*. Circulation 2004, 109: 1186-93.

92. Halcox, J.P., Nour, K.R., Zalos, G., Quyyumi, A.A. *Coronary vasodilation and improvement in endothelial dysfunction with endothelin ET(A) receptor blockade*. Circ Res 2001, 89: 969-76.

93. Wu, C., Chan, M.F., Stavros, F. et al. *Discovery of TBC11251, a potent, long acting, orally active endothelin receptor-A selective antagonist*. J Med Chem 1997, 40: 1690-7.

94. Tilton, R.G., Munsch, C.L., Sherwood, S.J. et al. *Attenuation of pulmonary vascular hypertension and cardiac hypertrophy with sitaxsentan sodium, an orally active ET(A) receptor antagonist*. Pulm Pharmacol Ther 2000, 13: 87-97.

95. Barst, R.J., Rich, S., Widlitz, A., Horn, E.M., McLaughlin, V., McFarlin, J. *Clinical efficacy of sitaxsentan, an endothelin-A receptor antagonist, in patients with pulmonary arterial hypertension: Open-label pilot study*. Chest 2002, 121: 1860-8.

96. Channick, R.N., Simonneau, G., Sitbon, O. et al. *Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: A randomised placebo-controlled study*. Lancet 2001, 358: 1119-23.

97. Rubin, L.J., Badesch, D.B., Barst, R.J. et al. *Bosentan therapy for pulmonary arterial hypertension*. New Engl J Med 2002, 346: 896-903.

98. Barst, R.J., Langleben, D., Frost, A. et al. *Sitaxsentan therapy for pulmonary arterial hypertension*. Am J Respir Crit Care Med 2004, 169: 441-7.

99. Langleben, D., Brock, T., Dixon, R., Barst, R. *STRIDE 1: Effects of the selective ETA receptor antagonist, sitaxsentan sodium, in a patient population with pulmonary arterial hypertension that meets traditional inclusion criteria of previous pulmonary arterial hypertension trials*. J Cardiovasc Pharmacol 2004, 44(Suppl. 1): S80-4.

100. Langleben, D., Hirsch, A.M., Shalit, E., Lesenko, L., Barst, R.J. *Sustained symptomatic, functional, and hemodynamic benefit with the selective endothelin-A receptor antagonist, sitaxsentan, in patients with pulmonary arterial hypertension: A 1-year follow-up study*. Chest 2004, 126: 1377-81.

101. Langleben, D., Hirsch, A., Shalit, E., Lesenko, L., Barst, R.J. *Sustained efficacy with the highly selective orally active endothelin-A receptor antagonist sitaxsentan after two years of therapy in patients with pulmonary arterial hypertension*. 101st Int Conf Am Thorac Soc (May 20-25, San Diego) 2005, A192.

102. *Tracleer® (bosentan tablets) US prescribing information*. Actelion Pharmaceuticals US, Inc, 2004. Available at: http:/www.tracleer.com/tralibrary/tralib004/DLD/tracleer_pi.pdf. Accessed February 1, 2005.

103. Benza, R.L., Mehta, S., Koegh, A., Lawrence, E.C., Oudiz, R.J., Barst, R.J., and the STRIDE-6 Study Group. *Sitaxsentan treatment for patients with pulmonary arterial hypertension discontinuing bosentan*. 101st Int Conf Am Thorac Soc (May 20-25, San Diego) 2005, A201.

104. Frost, A., Ivy, D. *Sitaxsentan for pulmonary arterial hypertension (PAH) patients with clinical deterioration or significantly abnormal liver function test on bosentan*. 101st Int Conf Am Thorac Soc (May 20-25, San Diego) 2005, A204.

105. Garces, P.C., Alford, K.L., Henry, N.S., Coyne, T.C. *Do liver function abnormalities with bosentan recur with sitaxsentan?* 101st Int Conf Am Thorac Soc (May 20-25, San Diego) 2005, A200.

106. Galié, N., Langleben, D., Badesch, D. et al. *STRIDE-2 trial: A placebo-controlled dose-ranging study for sitaxsentan in PAH*. ESC Cong (Sept 3-7, Stockholm) 2005, Abst 761.