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Review Article

Pulmonary Arterial Hypertension: Role of Ambrisentan

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ABSTRACT

Increasing numbers of experimental investigations and recently also of clinical trials strongly suggest an integral involvement of the endothelin (ET) system in the pathophysiology of a variety of disease states, mainly of the cardiovascular system.Ambrisentan (LU 208075)approved by the US Food and Drug Administration in 2007, a selective ETA-receptor antagonist, is an orally active diphenyl propionic acid derivative that was recently approved for treatment of pulmonary arterial hypertension (PAH) in patients with World Health Organization class II or III symptoms.. It has been shown to have a very promising efficacy to safety ratio in the initial clinical trials. Phase II and Phase III trials with ambrisentan in pulmonary arterial hypertension have been performed. Pulmonary arterial hypertension (PAH) is a rare and progressive disease of the pulmonary arterial circulation that is characterized by a progressive rise in pulmonary vascular resistance, eventually leading to right-heart failure and death. Endothelin (ET) is a potent vasoconstrictor with mitogenic, hypertrophic and pro-inflammatory properties. Therefore, blockade of ET receptors has been suggested as an attractive target in a number of acute and chronic cardiovascular indications, including pulmonary arterial hypertension (PAH), systemic hypertension, and heart failure. In Phase III clinical trials in patients with PAH, ambrisentan (2.5–10 mg orally once-daily) improved exercise capacity, time to clinical worsening, WHO functional class, and quality of life compared with placebo. This review discusses the endothelin family of proteins and receptors and their role in the pathophysiology of pulmonary hypertensive diseases.

Keywords: Pulmonary Hypertension, Endothelin, Endothelin receptor antagonist, Ambrisentan.

INTRODUCTION

Pulmonary Arterial Hypertension (PAH)

PAH differs from ordinary hypertension, which is high blood pressure throughout the body. In PAH, the high blood pressure is in the arteries between the heart and lungs. This causes the blood vessels that go from the heart to the lungs (or pulmonary arteries) to become tight, thick, and stiff. Typically, the pulmonary arteries are open and elastic, which creates no resistance to blood flow.[1] Pulmonary arterial hypertension (PAH) is defined as a blood pressure elevation in the vessels supplying the lung. It is characterized by vasoconstriction and vascular obstruction that eventually lead to increased pulmonary vascular resistance and right heart failure.[2]

As the disease progresses, patients experience a progressive rise in pulmonary artery pressure and pulmonary vascular resistance which leads to heart failure and premature death.[3,4,5] These high pressures make it difficult for the heart to pump blood through the lungs to be oxygenated.[6] It was previously defined as mean pulmonary artery pressure (MPAP)>25 mmHg at rest or >30 mmHg with exercise, but the definition was simplified to resting (MPAP) ≥25mmHg based on a review of current literature in 2008.[7] At the same time, pulmonary vascular tone may be increased and the ability of the pulmonary circulation to dilate or recruit underutilized vessels in response to increased flow is reduced.[8] These changes result in a progressive increase in pulmonary vascular resistance(PVR) that increases right ventricular afterload, reduces exercise tolerance and in most cases results in right ventricular failure.[9]

A key problem in treating PAH is the difficulty in confirming the diagnosis.[10] The early common symptoms include: breathlessness or dyspnea (present in 60 percent of patients), fatigue, dizziness, syncope or fainting (present in 13 percent of patients), weakness (present in 19 percent of patients), peripheral edema, and chest pains during physical activity. A key factor underlying the increase in pulmonary vascular resistance in PAH is thought to be pulmonary endothelial dysfunction, involving impaired production of vasodilators, such as nitric oxide (NO) and prostacyclin, and overexpression of vasoconstrictors, such asendothelin-1.[11]

The Venice 2003 Revised Classification system is[12]:

- WHO Group I - Pulmonary arterial hypertension
- WHO Group II - Pulmonary hypertension associated with left heart disease
- WHO Group III - Pulmonary hypertension associated with lung diseases and/orhypoxemia
- WHO Group IV - Pulmonary hypertension due to chronic thrombotic and/or embolicdisease
- WHO Group V – Miscellaneous.

Over the last 13 years, 6 drugs comprising 3 different drug classes have been approved for the treatment of PAH in the US (Table 1).

The five-group World Health Organization (WHO) classification scheme was last updated in 2008 at the 4th World Symposium on Pulmonary Hypertension (**Table 2**). Patients in WHO Group1 are classified as having pulmonary arterial hypertension (PAH), whereas patients in Groups 2 to 5 are classified as non-PAH or PH. Group 1 adds the criterion of pulmonary arterial wedge pressure of ≤15 mmHg and pulmonary vascular resistance (PVR) of ≥3

Wood's units. Group 1 also include sidiopathic and drug- and toxin-induced PAH.[13] The World Health Organization (WHO) classification of PAH is based on patients ' function, with class I signifying no limitation of usual daily physical activity, class II indicating mild limitation of physical activity, class III representing marked limitation of physical activity, and class IV indicating inability to perform any physical activity at rest, with possible signs of right-ventricular failure.[14]

The diagnosis of PAH is based on right heart catheterization demonstrating a mean pulmonary artery pressure (PAP) greater than 25 mm Hg with a normal left atrial pressure, as estimated by a pulmonary capillary wedge pressure (PCWP) less than 15 mm Hg, and pulmonary vascular resistance (PVR) greater than 3 Wood units.[15]

However, due to the rate of disease progression and the incurability of PAH, the **goals of therapy still focus upon:1**) preventing clinical worsening; and **2**) improving exercise capacity, functional class, hemodynamics, survival and quality of life. The understanding of the pathogenesisof PAH has increased substantially over the last few years,[16] as have treatment options, including prostaglandins and Potential phosphodiesterase inhibitors[17,18] and ET receptor antagonists.[19,20] There are several mechanisms through which ET receptor antagonists might improve PAH and/or right ventricular hypertrophy and failure. In addition to direct reduction of pulmonary artery tone through blocking ET receptors localized on vascular smooth muscle cells, a reduction of vascular media hypertrophy and thus a positive effect on vascular remodeling has been described in different preclinical experiments.[21,22] Progression of PAH is accompanied by impaired endothelial cell functioning that is characterized by an increase in vasoconstrictor and proliferative mediators and a decrease in vasodilator and antiproliferative mediators.[23]

Among the currently targeted therapies licensed in the U.S. for use in PAH are: the ETRAs (ambrisentan and bosentan), the PDE-5s (sildenafil and tadalafil), and the prostaglandins (epoprostenol, iloprost, and treprostinil). Clinical trials with these targeted therapies have demonstrated improvements in exercise capacity, hemodynamics, symptoms, and quality of life.[24]

Data suggesting a role of the endothelins in the pathophysiology of PAH include elevated circulating levels of endothelin-1 (ET-1) in patients with PAH and increased expression of ET-1 in plexi form lesions.[25] Occluding the distal pulmonary arteries of PAH patients. ET is one of many important neurohormones that play arole in the pathophysiology of PAH. ET-1 is a potent vasoconstrictor produced in the endothelium of pulmonary artery smooth muscle cells. ET_A receptors on the endothelial cells stimulate vasoconstriction and proliferation while the ET_B receptors are thought to stimulate the release of NO and PGI2, leading to vasodilation and counteracting the effects of ETA and ultimately ET-1. In PAH, lessET-1 is cleared. Higher levels of ET-1 correspond with more severe disease and prognosis.[26]

The Endothelins

In 1985, Hickey *et al* described an endothelium-derived contractile factor that was subsequently isolated and sequenced by Yanagisawa *et al* from porcine aortic endothelial cell cultures in 1988 and named endothelin.[27] The endothelins are a family of naturally occurring peptides that include endothelin1(ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). They are encoded by three different genes located on chromosomes 6, 1 and 20, respectively.[28] Endothelins are

mainly produced by vascular endothelial cells, and to a lesser extent by vascular smooth muscle cells, airway epithelial cells, macrophages, fibroblasts, cardiac myocytes, brain neurons, and pancreatic islet cells[29] and secreted from the vascular endothelium in response to numerous stimuli, including hypoxia, ischemia, shear stress, and growth factors and other neurohormones (eg,angiotensin II,norepinephrine).[30] All are 21 amino acid peptides containing 2 disulfide bonds.They are produced in endothelial cells lining the blood vessels.ET-1 acts primarily as a local autocrine and paracrine factor rather than as an endocrine hormone.[31] ET-1 was originally isolated from the supernatent of cultured porcine aortic endothelial calls, and is one of the most potent vasoconstrictor currently known.[32] Human ET-1 is Derived from the a 212 amino acid precursor protein (preproendothelin) which is cleaved by neutral endopeptidase (a furin like peptidase) to form a 38 amino acid pro ET-1 or bigendothelin. Big endothelin is essentially devoid of biologically activity, Endothelin converting enzyme-1 (ECE-1) converts big endothelin to mature endothelin peptide (ET-1). This conversion is physiologically important because ET-1 is a much more potent vasoconstrictor than big-ET-1 (about 140-fold) as shown in **Figure1.**[33]

Various evidence suggests that ET-1 might have a role in the pathogenesis and progression of PAH. For example, elevated levels of ET-1 have been found in patients with PAH, and PAH was found to be associated with the increased expression of ET-1 in vascular endothelial cells of pulmonary arteries.[34,35] ET-1 binds to 2 heptahelical G-protein-coupled receptors, named endothelin receptor-A (ETA) and endothelin receptor-B (ETB).The two subtypes of endothelin receptors have distinct ligand preference; ETA receptors are ET-1-selective, with an affinity order of ET-1≥ET-2 > ET-3, whereas ETB receptors exhibit similar affinities for the ETs.[36,37]

The intracellular mechanisms by which ETs induce smooth muscle contraction have not been fully elucidated.[38]These receptors are seven-transmembrane G protein-coupled membrane proteins. By associating with different Gα proteins, both receptors can activate different down stream protein kinases. ET-1 causes a transient increase in the intracellular concentration of free Ca^{2+} ions, followed by a sustained elevation of free Ca^{2+} ions for 30 to 60 minutes resulting in prolonged contraction.[39] The transient increase represents Ca2+ ions released from the intracellular pool of free Ca_2^{++} via activation of phospholipase C (PLC).[40] ET-A and ET-B to activate phospholipases resulting in elevation of inositol phosphate, diacylglycerol, eicosanoids, calcium.[41,42] ET-2 display similar pharmacology to ET-1, where as ET-3 is a weaker vasoconstrictor but more potent inhibitor of platelet aggregation .

Activation of ET-A and ET-B receptors on smooth muscle cells mediates the vasoconstrictive and mitogenic effects of ET. The mitogenic effects of ET-1 are mediated by the activation of protein kinase C secondary to increases in diacylglyceroland intracellular calcium, which in turn stimulate the production of cytokines and growth factors.[43] The ET-A receptors are primarily expressed on vascular smooth Muscle cells and cardiac myocytes and their activation mediates the vasoconstrictive and motohenic effect of Endothelin-1 (ET-1) ; in contrast, ETB receptors are localized predominantly on endothelial cells, and to alesser extent, on smooth muscle cells and on fibroblasts,[44] where they stimulate vasodilation via activation of endothelial cell Nitric oxide synthesis and ET-1 clearence via Receptor mediated endocytosis, although some ET_B receptor also located on smooth muscle

cells, where they mediates vasoconstriction and cellular proliferation.[45, 46]

Endothelin receptor A has 10 times more binding affinity for ET-1 and ET-2 than for ET-3 while ETB has equally potent affinities to all 3 endogenous endothelins.[47-49] Therefore, a selective ETA receptor antagonist may theoretically offer greater beneficial effects in PAH than antagonists of both ETA and ETB receptors.[50, 51]

Circulating plasma levels of ET-1 are raised in patients with PAH. Importantly, increased circulating levels of ET-1 correlate with increased right atrial pressure, increased pulmonary vascular resistance, decreased pulmonary artery oxygen saturation and increased mortality in patients with PAH.[52-55] Furthermore, there is an increase in the expression of ET-A and ET-B receptors in the lungs of patients with IPAH and PAH associated with congenital heart disease, although the relative proportion (60% ETA and 40% ETB) remains constant.[56]

Endothelin (ET) is among the strongest endogenous vasoconstrictors known and a potent mitogen. A rich body of experimental evidence suggests that ET contributes to vascular remodeling and end-organ damage in several cardiovascular conditions. Therefore, blockade of ET receptors has been suggested as an attractive target in a number of acute and chronic cardiovascular indications, including pulmonary arterial hypertension (PAH), systemic hypertension, and heart failure.

Subsequently, a large number of Phase II and III trials were conducted for a variety of disorders, including heart failure, cancer, pulmonary arterial hypertension, arterial hypertension, proteinuric renal disease, and autoimmune diseases.[57] Despite such intensive efforts, ERAs have been approved by the U.S. Food and Drug Administration for only two drugs and only two indications: bosentan and ambrisentan in pulmonary arterial hypertension,[58-60] A third ETRA – sitaxentan has been approved in the European Union, Australia, and Canada. These agents differ in their selectivity for the ETA and ETB receptors and also with regard to their pharmacokinetic properties. Endothelin receptor antagonism is a well-established approach to blocking the ET-1 system in PAH. Endothelin receptor antagonists (ERAs) are either ETA selective, such as sitaxsentan and ambrisentan, or nonselective for the ETA and ETB receptors, such as bosentan. ERAs block the activation of endothelin receptors on endothelial or smooth muscle cells, thereby inhibiting the vasoconstriction and cellular proliferation mediated by endothelin.[61-67]

Theoretically, selective ETA-receptor antagonists should be more effective in achieving vasodilation than non-selective ETA/ETB receptor antagonists, given the role played by ETB receptors in both vasodilation and ET-1 clearance.[68] Selective inhibition of ETA receptors may be preferential to non-selective receptor antagonism by permitting maintenance of vasodilator and clearance functions specific to ETB receptors on the endothelial cells, while preventing the vasoconstriction and cellular proliferation mediated by ETA .It is generally agreed that selective ETA antagonists display a more than 100- fold selectivity for the ETA receptor subtype. The selectivity, usually calculated from in vitro competitive receptor assays, can also be estimated in vivo on the basis of changes in circulating levels of ET-1. An increase in circulating ET-1 levels following administration of an ERA would reflect functional blockade of the ETB receptor.[69]

More recently, the ETA-selective, sulfonamide-class ERA sitaxsentan was approved in the EU. Ambrisentan, a non-sulfonamide, propanoic-acid class, ETA-selective ERA, is in late-stage clinical

development. Bosentan (Tracleer®) was approved in 2001 and has been moderately successful in improving quality of life for PAH sufferers, but is worrisome because of liver damage and interactions with other medications (oral contraceptives, statins, ketoconazole, amiodarone, glyburide, etc.).[70,71]

Ambrisentan therapy in pulmonary arterial hypertension

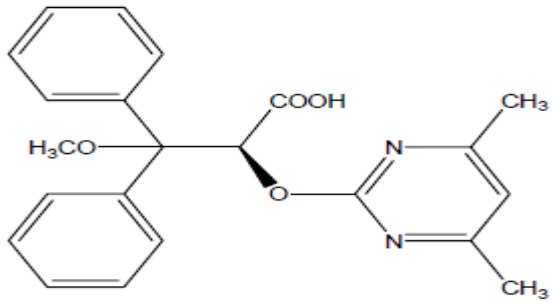
Chemistry and Development of Ambrisentan

Several different structural classes of ERAs, ranging from peptidic and peptidomimetic structures to small organic molecules suitable for oral administration have been discovered within the last decade. Bosentan, one of the first orally active compounds with an antagonistic effect of ETA and ETB, is a tetra-substituted pyrimidine.[72] It was the first ERA approved for treatment of PAH in Europe and the US and its therapeutic efficacy has been demonstrated in several randomized, double-blind, placebo-controlled studies.[73,74] Ambrisentan was approved for sale by the U.S. Food and Drug Administration (FDA) on June 15, 2007 for the once-daily treatment of pulmonary arterial hypertension.[75-77] It was later approved by the European Medicines Agency for use in the EU on April 2008.[78] Ambrisentan had previously been designated an orphan drug by both the FDA and the European Commission, in August 2004 and May 2005 respectively.

The initial 3,3-diphenyl propionic acid based ERAs from BASF/Knoll were discovered by screening of BASF's chemical library for compounds that bind to recombinant human ETA receptor revealed two diphenyl propionic acid derivates, which were originally designed as herbicides.[79] Modification of this structure with the aim to simplify the structure and to enhance the binding activity led to a series of potent orally available ETA-selective receptor antagonists.[80] The active enantiomer of the first compound was named LU 135252, which was studied clinically as darusentan (Fig. 2). Slight modification of the structure of darusentan led to a similar compound, LU 208075, which was named ambrisentan.

Finally, ambrisentan is the latest endothelin antagonist to enter the market. The orphan drug is indicated for the treatment of pulmonary arterial hypertension and is available only through a limited distribution system.[81]

LETAIRIS is the brand name for ambrisentan, an endothelin receptor antagonist that is selective for the endothelin type-A (ETA) receptor. The chemical name of ambrisentan is (+)-(2S)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid. It has a molecular formula of C22H22N2O4 and a molecular weight of 378.42. It contains a single chiral center determined to be the (S) configuration and has the following structural formula:



Clinical data

Clinical trials with ambrisentan have been performed since 2001 (**Table 3**).However, the initial Phase I trials in patients with cardiovascular disease and renal failure were mentioned only in the reports of Abbott Laboratories but no data from these trials were published. Similarly,

the initial Phase II trials, conducted by Myogen since 2001, in patients with hypertension, renal failure, cardiac failure, or cardiovascular disease were not reported in the literature.

The first clinical data from the Phase II trial with ambrisentan in patients with PAH wasmade available in September 2003 (Myogen website) and published in 2005. In thisdose-blinded randomized trial, 64 patients suffering from PAH grades WHO II or III were treated with ambrisentan (1, 2.5, 5, or 10 mgd), initially for 12 weeks. Afterwards, 54 of these patients participated in an optional open labeled extension period of 12 weeks. At the end of this extension period the medication was further continued in 44 patients. Furthermore, the 6 minutes walk distance,which is a standard functional parameter in PAH, significantly increased in about 30% of patients during the initial 12 weeks and in 50% during the subsequent open label period. An improvement of the hemodynamic parameters was also observed.

The safety and efficacy of ambrisentan were assessed in two 12-week, randomized, doubleblind, placebo-controlled studies involving 393 patients with PAH. Ambrisentan or placebo was added to current therapy, which could have included a combination of anticoagulants, diuretics, calcium-channel blockers or digoxin, but not epoprostenol, treprostinil, iloprost, bosentan or sildenafil. One trial (ARIES-1) compared once-daily oral doses of 5 mg and 10 mg ambrisentan to placebo, and another (ARIES-2) compared once-daily doses of 2.5 mg and 5 mg ambrisentan to placebo. The primary end point in both studies was a 6-minute walk distance.[82]

Two 12-week, randomized, double-blind, placebo-controlled, multicenter studies wereconducted in 393 patients with PAH (WHO Group 1). The two studies were identical indesign except for the doses of LETAIRIS and the geographic region of the investigational sites. ARIES-1 compared once-daily doses of 5 mg and 10 mg LETAIRIS to placebo, while ARIES-2 compared once-daily doses of 2.5 mg and 5 mg LETAIRIS to placebo. In both studies, LETAIRIS or placebo was added to current therapy, which could have included a combination of anticoagulants, diuretics, calcium channel blockers, or digoxin, but not epoprostenol, treprostinil, iloprost, bosentan, or sildenafil. The primary study endpoint was 6-minute walk distance. In addition, clinical worsening, WHO functional class, dyspnea, and SF-36® Health Survey were assessed.

Patients had idiopathic or heritable PAH (64%) or PAH associated with connectivetissue diseases (32%), HIV infection (3%), or anorexigen use (1%). There were nopatients with PAH associated with congenital heart disease. Patients had WHO functional class I (2%), II (38%), III (55%), or IV (5%) symptoms at baseline. The mean age of patients was 50 years, 79% of patients were female, and 77% were Caucasian. Submaximal Exercise Ability Results of the 6-minute walk distance at 12 weeks for the ARIES-1 studies are shown in **Table 4 and Figure 3**.

Although data describing the selectivity of ambrisentan for the ETA receptor vary between 29:1 [83] to >4000:1 [84] depending on the assay cited, the drug is considered a selective ETA receptor antagonist.[85,86] Ambrisentan is a relatively selective antagonist of the ETA receptor. A Phase-II dose-ranging study supported the efficacy and safety of ambrisentan in patients with PAH, and subsequently two pivotal Phase-III clinical trials of ambrisentan in PAH confirmed these findings. Ambrisentan belongs to the group of carboxylic ERAs which unlike sulfonamide-based ERAs – are devoid of hepatotoxicity. In fact, patients on ERAs

with elevated liver function tests on sulfonamide-based ERAs such as bosentan or sitaxentan have been successfully switched to ambrisentan.[87,88] Positive results were recently published from a phase II double-blind, dose-ranging study of ambrisentan 1mg, 2.5mg, 5mg or 10mg once-daily for six months in patients with PAH.

Ambrisentan is indicated for use in the treatment of pulmonary arterial hypertension (PAH) World Health Organization (WHO) group 1 in patients with WHO class II or III symptoms to improve exercise capacity and delay clinical worsening.[89] High-throughput screening led to the identification of nonpeptidic propionic acid derivatives with potent antagonist activity against the ETA receptor7. Ambrisentan is a propionic acid derivative that has a high selectivity for the ETA receptor versus the ETB receptor [90] whereas the other FDA-approved endothelin-receptor antagonist, bosentan, affects both receptors.[91]

Ambrisentan is developed as an ETA receptor antagonist with a high potency, high oral bioavailability, and long half life suitable for once a day dosing.[92] Ambrisentan is available as 5 mg and 10 mg film-coated tablets for once daily oral administration. The tablets include the following inactive ingredients: croscarmellose sodium, lactose monohydrate, magnesium stearate and microcrystalline cellulose. Ambrisentan is a high affinity (Ki=0.011 nM) ETA receptor antagonist with a high selectivity for the ETA versus ETB receptor (>4000-fold). The clinical impact of high selectivity for ETA is not known. Ambrisentan has slightly more selectivity for the ET-A compared with the ET-B receptor (selectivity ratio, 77:1) than bosentan (20:1); however, the clinical relevance of this difference in selectivity is unknown. At therapeutically relevant plasma concentrations in PAH, ambrisentan has higher receptor occupancy with ETA (90%), than ETB (10%). Despite this, the reported selectivity of ambrisentan for ETA of only 77/1,[93] is somewhat less than the generally accepted ratio needed for the qualification as a selective ETA receptor antagonist. Furthermore, significant increases in plasma ET-1 levels have been reported 2 hours after ingestion of ambrisentan suggesting at least some functional antagonism of ETB in vivo.[94,95]

Pharmacokinetics of ambrisentan

The pharmacokinetics of ambrisentan (S-ambrisentan) in healthy subjects are dose proportional. The bioavailability of orally administered ambrisentan in dogs is about 90% and its duration of action is longer than 6 hr. In a Phase II clinical investigation, in patients suffering from PAH, plasma levels of ambrisentan increased rapidly after oral administration of the drug and reached C-max between 1.7 and 3.3 hr. The mean elimination half-time was between 9 and 15 hr. In vitro studies indicate that ambrisentan is a substrate of P-gp. Ambrisentan is highly bound to plasma proteins (99%). The elimination of ambrisentan is predominantly by non-renal pathways, but the relative contributions of metabolism and biliary elimination have not been well characterized. The mean oral clearance of ambrisentan is 38 mL/min and 19 mL/min in healthy subjects and in PAH patients, respectively.

The chemical composition of ambrisentan, also known as LU 208075 or BSF 208075, is $C_{22}H_{22}N_2O_4$, and its molecular weight is 378.4 g/mol. Another significant difference from bosentan is the drug interaction profile. Bosentan is both a substrate and an inducer of CYP2C9 and CYP3A4, while ambrisentan is a substrate only for CYP2C19 and CYP3A4, as well as

P-glycoprotein and organic anion transport protein.

Ambrisentan is a specific inhibitor of endothelin A receptors, while bosentan inhibits both endothelin A and endothelin B; although selectivity may have theoretical advantages, these advantages remain to be proven.[96,97] Ambrisentan is a white to off-white, crystalline solid. It is a carboxylic acid with a pKa of 4.0. Ambrisentan is practically insoluble in water and in aqueous solutions at low pH. Solubility increases in aqueous solutions at higher pH. In the solid state ambrisentan is very stable, is not hygroscopic, and is not light sensitive. Steady-state is achieved after 3–4 days of once-daily oral dosing with ambrisentan, and the pharmacokinetics of multiple doses (ie, steady-state ambrisentan) are consistent with observations after a single dose.[98] The steady-state elimination half-life of ambrisentan in PAH patients is approximately 15 hours for the 5 mg dose, providing the rationale for once-daily dosing.[99] In vitro data indicate that the metabolism of ambrisentan is affected by cytochrome P450 (CYP) 3A4 and 2C19 isozymes, as well as by strong inhibitors of P-glycoprotein.[100]

Drug Interaction

Potential interactions have not been well characterized. Based on in vitro data, interactions with P-glycoprotein, OATP, CYP3A4, and CYP2C19 inhibitors, and UGTs are anticipated. Current sulfonamide-class ERAs developed for the treatment of PAH are associated with potentially significant drug-drug interactions. Bosentan induces the cytochrome P450 isoenzymes CYP2C9 and CYP3A4 and may decrease the systemic exposure of other drugs that share this metabolic pathway.[101] Similarly, sitaxsentan inhibits the activity of CYP2C9, thereby increasing systemic exposure to drugs metabolized by this cytochromeP450 isozyme.[102] Ambrisentan should be used with caution in patients receiving concomitant cyclosporine. Cyclosporine is a strong inhibitor of P-glycoprotein, OATP, and CYP3A4, therefore, ambrisentan exposure may be increased with concomitant cyclosporine administration. Caution is also advised when ambrisentan is coadministered with strong CYP3A and 2C19 inhibitors, or inducers of P-glycoprotein, CYP-450 enzymes, or UGTs.

The interaction of ambrisentan and warfarin was evaluated in 22 healthy volunteers, who were given a single 25-mg oral dose of warfarin before and after 8 days of oral ambrisentan 10 mg/d.[103] A 2-period crossover study was conducted in 19healthy adults to evaluate the interaction between sildenafil and ambrisentan.[104] During the first period, 19 healthy volunteers were given an oral dose of ambrisentan 10 mg before and after receiving 7 days of oral sildenafil 20 mg TID. During the second period of the study, the same volunteers were given a single oral dose of sildenafil 20 mg before and after receiving 7 days of oral ambrisentan 10 mg/d. The Cmax and AUC of sildenafil and ambrisentan were not significantly changed. No dose adjustment is required whenthe 2 agents are coadministered.

In a Phase 2, dose-ranging study, prothrombin time, international normalized ratio, and anticoagulant dose were unaffected by ambrisentan treatment.[105] Similar results were reported for warfarin-type anticoagulant dosing in the Phase 3 ambrisentan trials.[106] These data suggest that dosage adjustmentof warfarin is not needed during concomitant ambrisentan therapy.

Ambrisentan received FDA approval in June 2007. It is available as 5 and 10 mg tablets packaged in 30-count blister packs. Ambrisentan tablets should be stored at 25°C (77°F), with excursions permitted between 15° and 30°C (59° and 86°F).

Contraindication

Ambrisentan is contraindicated in patients who are pregnant or may become pregnant.Ambrisentan may cause fetal harm when administered to a pregnant woman. Teratogenicity is a class effect of endothelin receptor antagonists. Ambrisentan is in Pregnancy Category X. Teratogenicity has been observed in animal models and is a class effect of endothelin receptor antagonists. In animal studies, ambrisentan was teratogenic at oral doses of ≥15 mg/kg per day in rats and ≥7 mg/kg per day in rabbits.[107] It is recommended that women who are taking ambrisentan not breastfeed.

Warnings and Precautions

Hepatic toxicity has been observed with ambrisentan, bosentan, and sitaxsentan. Ambrisentan has been observed to cause elevation of liver aminotransferases to at least 3 times the upper limit of normal (ULN) in 0.8% of patients in 12-week ambrisentan studies and 2.8% of patients in long-term, open-label studies lasting up to 1 year. Ambrisentan should be avoided in patients with elevated aminotransferases (greater than three times the ULN) at baseline. Reductions in hemoglobin and hematocrit have been observed within the first few weeks of ambrisentan therapy. More than 15% reductions in hemoglobin from baseline resulting in levels below the lower limit of normal occurred in 7% of patients treated with ambrisentan in clinical trials (10% in the patients treated with 10 mg) compared with 4% of placebo-treated patients. Hemoglobin monitoring is recommended during ambrisentan therapy. Mild to moderate peripheral edema has been observed during ambrisentan therapy. If clinically significant fluid retention develops, with or without associated weight gain, further evaluation should be undertaken to determine the cause, such as LETAIRIS or underlying heart failure, and the possible need for specific treatment or discontinuation of LETAIRIS therapy. Ambrisentan use is not recommended in patients with moderate or severe hepatic function impairment. Ambrisentan should be used with caution in patients with mild hepatic function impairment.

Adverse Reactions

The most frequently reported adverse reactions included peripheral edema, nasal congestion, sinusitis, flushing, palpitations, abdominal pain, and constipation. The majority of the adverse reactions were classified as mild to moderate in severity and only the incidence of nasal congestion was dose dependent.

Safety

Ambrisentan was generally safe and well tolerated in allthe PAH clinical studies. The most frequently reportedadverse events during the Phase 2, dose-ranging study were peripheral edema, nasal congestion, upper respiratory tract infection, headache, flushing, and nausea and did not appear to be dose related. Ambrisentan was well tolerated throughout the 1-year extension study, with no emergent safety signals apparent during long-term therapy.[108]

Liver abnormalities have been associated with the sulfonamide- class ERAs and to date necessitate monthly liver function testing (LFT).Alteration of liver enzymes (mostly aminotransaminases) occured only in a few patients treated with ambrisentan. The incidence of this side effect with ambrisentan appearsto be lower than with ET receptor antagonists with a pyrimidine structure, like bosentan.[109-111] Also, no clinically relevant increase in liver enzymes was reported in the clinical trials with darusentan. It is, therefore, conceivable that in patients treated with diphenyl propionic acid derivatives, like ambrisentan

or darusentan, this effect is caused by the cytochrome p450 independent metabolism. Serious events of liver toxicity in patients treated with ET receptor antagonists have only been reported in a few cases and after long term therapy.

In comparison with other ET-receptor antagonists ambrisentan appeared to have a favorable efficacy to safety ratio in patients with PAH. In contrast to bosentan, ambrisentan has demonstrated a lower incidence of acute hepatotoxicity in the Phase 2 and 3 clinical trials. During the Phase 2, 24-week study, 2 (3%) patients experienced elevations in hepatic aminotransferases >3 × ULN that required dose reduction or drug discontinuation; an additional 2 patients had isolated elevations that were unconfirmed upon retest and required no change in treatment.

Summary

In summary, ambrisentan, an ETA-selective receptor antagonist, seems to improve exercise capacity, symptoms, and hemodynamics in patients with WHO class II to III PAH. Ambrisentan may have a very favorable efficacy-to-safety ratio in patients with PAH, including a low incidence and severity of serum aminotransferase abnormalities that does not seem to be dose-dependent. Phase III placebo-controlled trials are currently on going to confirm these initial results. A direct comparison of selective ETA- with dual ETAB-receptor antagonists is necessary for selection of the best treatment. Endothelin 1 is clearly a key mediator in the pathogenesis of PAH. Advances in basic knowledge of its role in pulmonary vascular remodelling have led to translational clinical studies in man. Thus, endothelin receptor antagonists are now a cornerstone in the management of this devastating condition. However, over the lifetimeof the clinical studies to date, there are no obvious advantages of differential receptor blockade (i.e. ETA vs. ETA/ETB). Finally, the potential benefit of endothelin converting enzyme inhibition has yet to be explored in PAH.

Conclusion

Ambrisentan offers an alternative to bosentan therapy. Ambrisentan is dosed once daily, rather than twice daily like bosentan. It may be associated with a lower incidence of liver toxicity; however, close monitoring is still necessary. The choice of drug is dependent on a variety of factors, including the approval status, route of administration, side effect profile, patient preference, and the physician's experience and clinical judgment. Ambrisentan is a selective ETA receptor antagonist that appears to provide significant clinical benefit in the treatment of patients with PAH. In Phase 2 and 3 clinical trials, ambrisentan improved exercise capacity, dyspnea, time to clinical worsening, WHO functional class, quality of life, and cardiopulmonary hemodynamic parameters.

Ambrisentan has an improved safety profile compared with sulfonamide-class ERAs with respect to the potential for hepatic toxicity and the potential for drug-drug interactions with agents metabolized by P450 enzymes such as warfarin and sildenafil. Available clinical data suggest that ambrisentan will be successful in the treatment of PAH. Several experimental and clinical trials will be necessary to determine the optimal ET-receptor antagonist profile for the specific pathological application.

Expert opinion

The pathogenesis of pulmonary hypertension (PH) is complex and multifactorial. Increased pulmonary arterial pressure (PAP) in patients with PH probably results from a combination of pulmonary vasoconstriction, inward vascular wall

remodelling and in situ thrombosis. The central role of endothelial dysfunction in the initiation and progression of PH resulting in the altered production of endothelial mediators has been increasingly recognised in PH. Of those local mediators, nitric oxide (NO), prostacyclin (PGI2), serotonin (5-HT) and endothelin (ET) are among the best studied and most commonly implicated in the pathogenesis of pulmonary arterial hypertension (PAH).Pulmonary arterial hypertension is a progressive and generally incurable disease that afflicts approximately 117 000 women and men in the United States and approximately the same number in the EU.[112] However, despite an overall poor prognosis, long-term survival and higher quality of life are increasingly achievable in patients with PAH. Advances in treatment have closely followed identification and characterization of disease pathways such that novel treatments are available that affect the prostacyclin, nitric oxide, and endothelin pathways.

Endothelin receptor antagonists have emerged as a cornerstone of therapy for patients with PAH when used either as monotherapy or, increasingly, as an integral component of novel combination regimens.[113-114] Because some drugs have a narrow therapeutic window or, alternatively, have safety concerns, coadministered agents that exhibit pharmacologic interactions may limit the effectiveness or exacerbate drug toxicity. Inthis context, potential drug-drug interactions are increasingly important in selecting a treatment for use in combination. Given the significant role that ET-1 seems to play in the pathobiology of PAH, there is a strong rationale for the use of ERAs in the treatment of PAH. A selective ETA receptor antagonist may afford us the opportunity of blocking the actions of ET-1 at the predominant vasoconstrictor receptor subtype, that is, ETA receptor, while permitting ongoing stimulation of the vasodilatory ETB receptor on endothelial cells, in addition to preserving ET-1 pulmonary clearance.

The ETA-selective ERA ambrisentan holds promise in advancing both the safety and convenience of oral PAH treatment. Ambrisentan, a once-daily oral treatment, does not induce or inhibit cytochrome P450 enzymes; therefore, ambrisentan may be preferable to other currently available ERAs when used in conjunction with other medications prescribed for patients with PAH. In clinical trials with ambrisentan, there appears to be a low risk for acute hepatotoxicity. An oral once-daily agent with a low risk for liver function abnormalities and drug–drug interactions could confer advantages to the agent's use in combination regimens, a current area of active investigation and optimism.

Improved understanding of the cellular processes involved in the pathogenesis of PAH has allowed for identification of several prospective targets for pharmacologic intervention, including vasoactive intestinal peptide (VIP), platelet-derived growth factor (PDGF), voltage-gated potassium channels, 5-hydroxytryptamine (serotonin) transporter (SERT) and vascular endothelial growth factor (VEGF), tyrosine kinase inhibitors, Rho-kinase inhibitors, statins, drug combinations and apoptotic therapies such as dichloroacetate among others. It is not known which, if any, of these approaches will benefit patients with PAH, and it is clear that well-designed, controlled clinical trials, similar to those used to test ambrisentan, will be required to measure benefits and complications. Until that time, ambrisentan is a new drug with an excellent benefit-to-risk profile and should find significant usage in the treatment of PAH.

Experimental studies have shown preliminary favourable effects of gene and

stem cell therapy in animal models of pulmonary hypertension. First experiences with these innovative approaches in patients with PAH have been planned. By identifying the genes and gene variants that determine individual disease susceptibility, we might be able to identify patients in preclinical stages of disease as well as allow for individualized therapies that are most efficacious and least likely to cause side effects. Ultimately, as these novel therapeutic options are developed, individualized treatment regimens will evolve. We hope that by further increasing our understanding of the pathobiology of PAH, we will one day be able to prevent and cure this disease.

REFERENCES

1. Hielsen LH, Lokkegaard E, Andreasen AH, *et al*. 2008.Using prescription registries to define continuous drug use: how to fill gaps between prescriptions. Pharmaco epidemiology and Drug Safety,17(4):384-388.
2. Newman JH, Fanburg BL, Archer SL, *et al*. 2004. For the National Heart, Lung, and Blood Institute/Office of Rare Diseases. Pulmonary arterial hypertension: Future directions: Report of a National Heart, Lung, and Blood Institute/Office of Rare Diseases workshop. *Circulation*,109:2947–2952.
3. National Institute for Health and Clinical Excellence. Appraisal of the drug treatments in Pulmonary Arterial Hypertension (PH).Pulmonary Arterial Hypertension Association UK; 2007.
4. Letairis (ambrisentan). Learn about Letairis. Available on: http://www.letairis.com. Accessed on October 14, 2010.
5. Humbert M, Khaltaev N, Bousquet J, *et al*. 2007. Pulmonary Hypertension: From an Orphan Disease to a Public Health Problem. Chest,312:365-467.
6. Pulmonary Hypertension Association: about PAH. Available at: http://www.phassociation.org/page.aspx?pid=538. Accessed October 13, 2010.
7. Badesch DB, Champion HC, Sanchez MA, *et al*. 2009.Diagnosis and assessment of pulmonary arterial hypertension. *J Am Coll Cardiol*,54(suppl 1):S55-S66.
8. Chan SY, Loscalzo J. 2008. Pathogenic mechanisms of pulmonary arterial hypertension. *J Mol Cell Cardiol*, 44:14–30.
9. Granton J, Moric J. 2008. Pulmonary vasodilators–treating the right ventricle. *Anesthesiol Clin*, 26:337–53, vii.
10. Pulmonary Hypertension Association: Newly Diagnosed. Available at:http://www.phassociation.org/page.aspx?pid=538. Accessed October 13, 2010.
11. Humbert, M. *et al*.2004.Treatment of pulmonary arterialhypertension. *N. Engl. J. Med*,351:1425–1436.
12. **[**No authors listed] Proceedings of the 3rd World Symposium on Pulmonary Arterial Hypertension. Venice,Italy, June 23-25, 2003. *J Am Coll Cardiol*. 2004 Jun 16;43(12 Suppl S):1S-90S.
13. Simonneau G, Robbins IM, Beghetti M, *et al*. 2009.Updated clinicalclassification of pulmonary hypertension. *J Am Coll Cardiol*,54(suppl 1):S43-S54.
14. Simonneau G, Galiè N, Rubin LJ, *et al*.2004.Clinical classification of pulmonary hypertension.*J Am CollCardiol*,43 (Suppl S):5S–12S.
15. Barst RJ, McGoon M, Torbicki A, *et al*. 2004.Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol*,43(12 Suppl S):40S–47S.
16. Hoeper MM, Galie N, Simonneau G, *et al*. 2002. New treatments for pulmonary arterial hypertension. *Am J Respir Crit Care Med*,165:1209–1216.
17. Ghofrani HA,Wiedemann R, Rose F, *et al*. 2002. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomized controlled trial. *Lancet*, 360:895–900.
18. Ghofrani HA, Rose F, Schermuly RT, *et al*. 2003. Oral sildenafil as long-term adjunct therapy to inhaled iloprost in severe pulmonary arterial hypertension. *J Am CollCardiol*,42:158–164.

19. Channick RN, Simonneau G, Sitbon O, *et al*. 2001. Effects of the dualendothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomized placebo-controlled study. *Lancet*,358:1119–1123.
20. Rubin LJ, Badesch DB, Barst RJ, *et al*. 2002.Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med*,346:896–903.
21. Galie N, Manes A, Branzi A.2004. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res*, 61:227–237.
22. Michel RP, Langleben D, Dupuis J. 2003.The endothelin system in pulmonary hypertension. *Can J Physiol Pharmacol*,81: 542–554.
23. Rich S. 2001. Pulmonary Hypertension. In Braunwald E, Zipes DP, Libby P(eds). Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia, PA.*WB Saunders*, 1912–29.
24. Hyduk A, Croft JB, Ayala C, Zheng Z, *et al*. 1980-2002. Pulmonary Hypertension Surveillance-United States, MMWR. Surveillance Summaries, November 11, 2005/54(SS05);128. Availableathttp://www.cdc.gov/mmwr/preview/mmwrhtml/ss5405a1.htm.AccessedNovember3,2010.
25. Matsui K, Takano Y, Yu ZX, *et al*. 2002. Immuno histochemical study of endothelin-1 and matrix metallo proteinases in plexogenic pulmonary arteriopathy. *Pathol Res Pract*, 198:403–12.
26. McLaughlin VV, Archer SL, Badesch DB, *et al*. 2009-2011. ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. *Circulation*.119:2250-2294.
27. Yanagisawa M, Kurihara H, Kimura S, *et al*. 1988. A novel potent vaso constrictor peptide produced by vascular endothelial cells. *Nature*, 332:411–5.
28. Kawanabe Y, Nauli SM.2011.Endothelin. *Cell Mol Life Sci*,68:195–203.\
29. Price LC, Howard LS. 2008.Endothelin receptor antagonists for pulmonary arterial hypertension: rationale and place in therapy. *Am J Cardio vasc Drugs*, 8:171–85.
30. Levin ER.1995.Endothelins.*N Engl J Med*, 333:356–363.
31. Wagner OF, Christ G, Wojta J, Vierhapper H, Parzer, S, Nowotny PJ, *et al*.1992. Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem*, 267:16066–16068.
32. Masaki T. and Yanagisawa M. 1992. Physiology & Pharmacology of endothelins. *Medicinal Research Reviews*, 12:391-421.
33. Rubanyi GM, & Polokoff MA. 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol Rev*, 46:325– 415.
34. Remuzzi G, Perico N, and Benigni A.2002. New therapeutics that antagonize endothelin: promises and frustrations. Nature Rev. *Drug Discov*.,1: 986–1001.
35. Giaid, A. *et al*. Expression of endothelin-1 in the lungs of patients with primary pulmonary hypertension. *N. Engl. J. Med*. 328, 1732-1739 (1993).
36. Ogawa Y, Nakao K, Arai H, Nakagawa O, Hosoda K, Suga S, *et al*. Molecular cloning of a non-isopeptide-selective human endothelin receptor. *Biochem Biophys Res Commun* 1991;178:248–55.
37. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* 1990;348:730–2.
38. Yanagisawa H, Hammer RE, Richardson JA, *et al*. 2000. Disruption of ECE-1 and ECE-2 reveals a role for endothelin-converting enzyme-2 in murine cardiac development. *J Clin Invest*, 105:1373–82.
39. Masaki T, Miwa S, Sawamura T, Ninomiya H, *et al*. 1999. Subcellular mechanisms of endothelin action in vascular system. *Eur J Pharmacol*, 375:133–8.
40. Miwa S, Iwamuro Y, Zhang XF, *et al*. 1999. Ca2+ entry channels in rat thoracic aortic smooth muscle cells activated by endothelin-1. *Jpn J Pharmacol*, 80:281–8.

41. Naylor,W.G.(1990). Endothelin : Isoforms, binding sites and possible implication in pathology. *Trends Pharmacol. Sci*.11, 96-99.
42. Cernacek, P. and Stewart, D.J.(1989). Immunoreactiveendothelin in human plasma; marked elevations in patients in cardiogenic shock. Biochem. *Bio Phys. Res.commun*. 161, 562-567.
43. Ohlstein EH, Arleth A, Bryan H, *et al*. 1992. The selective endothelin ETA receptor antagonist BQ123 antagonizes endothelin-1-mediated mitogenesis. *Eur J Pharmacol*, 225:347–50.
44. Dupuis J. 2001. Endothelin-receptor antagonists in pulmonary hypertension. *Lancet*, 358:1113–4.
45. Galie N, Manes N, Branzi A, The endothelin system in pulmonary hypertension. *Cardiovasc. Res*. 2004: 61(2): 227-37.
46. Reinhart GA, Preusser LC, Opgenorth TJ, Wegner CD, Cox BF. Endothelin and ET_A receptor in long-term arterial pressure homeostasis in conscious non human primates.*Am. J Physiol Regul Inter Comp Physiol.* 2000: 279(5): R1701-6.
47. Watanabe, H., Miyazaki, H., Kondoh, M., Masuda, Y., Kimura, S., Yanagisawa, M., *et al*. (1989). Two distinct types of endothelin receptors are present on chick cardiac membranes. *Biochem Biophys Res Commun* 161, 1252– 1259.
48. Takuwa, Y., Masaki, T., & Yamashita, K. (1990). The effects of the endothelin family peptides on cultured osteoblastic cells from rat calvariae. *Biochem Biophys Res Commun* 170, 998–1005.
49. Williams Jr., D. L., Jones, K. L., Pettibone, D. J., Lis, E. V., &Clineschmidt, B. V. (1991). Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. *Biochem Biophys Res Commun* 175, 556– 561.
50. Jacobs A, Preston IR, Gomberg- Maitland M. Endothelin receptor antagonism in pulmonary arterial hypertensiona role for selective ETA inhibition. *Curr Med Res Opin*. 2006; 22(12):2567-2574.
51. Riechers H, Albrecht HP, Amberg W, *et al*. Discovery and optimization of a novel class of orally active nonpeptidicendothelin-A receptor antagonists. *J Med Chem* 1996;39:2123–2128.
52. Cacoub P, Dorent R, Nataf P, Carayon A. Endothelin-1 in pulmonary hypertension. *N Engl J Med* 1993;329:1967–8.
53. Galie N, Ussia G, Passarelli P, Parlangeli R, Branzi A, Magnani B. Role of pharmacologic tests in the treatment of primary pulmonary hypertension. *Am J Cardiol* 1995;75:55A–62A.
54. Cacoub P, Dorent R, Nataf P, Carayon A, Riquet M, Noe E, *et al*. Endothelin-1 in the lungs of patients with pulmonary hypertension. *Cardiovasc Res* 1997;33:196–200.
55. Nootens M, Kaufmann E, Rector T, Toher C, Judd D, Francis GS, *et al*. Neurohormonal activation in patients with right ventricular failure from pulmonary hypertension: relation to hemodynamic variables and endothelin levels. *J Am CollCardiol* 1995;26:1581–5.
56. Davie N, Haleen SJ, Upton PD, Polak JM, Yacoub MH, Morrell NW, *et al*. ET(A) and ET(B) receptors modulate the proliferation of human pulmonary artery smooth muscle cells. *Am J RespirCrit Care Med* 2002;165:398–405.
57. Battistini B, Berthiaume N, Kelland NF, Webb DJ, Kohan DE. Profile of past and current clinical trials involving endothelin receptor antagonists: the novel "-sentan" class of drug. *ExpBiol Med (Maywood)* 2006;231(6):653–95.
58. Rubin, L. J. *et al*. Bosentan therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* 346, 896–903 (2002).
59. Galie N, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, *et al*. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2.*Circulation* 2008a;117(23):3010–9.
60. Galie N, Rubin L, Hoeper M, Jansa P, Al-Hiti H, Meyer G, *et al*. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study):

a double-blind, randomised controlled trial. *Lancet* 2008b;371(9630):2093–100.
61. Badesch D B, McLaughlin V V, Delcroix M *et al*., "Prostanoid therapy for pulmonary arterial hypertension", *J Am Coll Cardiol* (2004);43: pp. 56S–61S.
62. Fagan K A, McMurtry I F, Rodman D M, "Role of endothelin-1 in lung disease", *Respir Res* (2001);2: pp. 90–101.
63. Farber H W, Loscalzo J, "Pulmonary arterial hypertension", *N Engl J Med* (2004);351: pp. 1,655–1,665.
64. Galie N, Badesch D, Oudiz R *et al*., "Ambrisentan therapy for pulmonary arterial hypertension", *J Am CollCardiol* (2005);46: pp. 529–535.
65. Griendling K K, Harrison D G H, Alexander R H, "Molecular and cellular biology of blood vessels", in Hurst's the Heart, *McGraw-Hill* (2001); pp. 127–146.
66. Humbert M, Sitbon O, Simonneau G, "Treatment of pulmonary arterial hypertension", *N Engl J Med* (2004);351: pp. 1,425–1,436.
67. Newman J H, Fanburg B L, Archer S L *et al*., "Pulmonary arterial hypertension: future directions: report of a National Heart, Lung and Blood Institute/Office of Rare Diseases workshop", *Circulation* (2004);109: pp. 2,947–2,952.
68. Christian, F. O.; Ralf, E.; Wilhelm, K.; David, P. Eur. *Heart J.* 2008, 29, 1936.
69. Holm, P., & Franco-Cereceda, A. (1999). Haemodynamic influence and endothelin-1 plasma concentrations by selective or non-selective endothelin receptor antagonists in the pig in vivo. *Acta Physiol Scand* 165, 163– 168.
70. Diller GP, Dimopoulos K, Kaya MG, Harries C, Uebing A, Li W, Koltsida E, Gibbs JS, Gatzoulis MA. Longterm safety, tolerability and efficacy of bosentan in adults with pulmonary arterial hypertension associated with congenital heart disease. *Heart*. 2007 Aug;93(8):974-6.
71. McLaughlin VV. Survival in patients with pulmonary arterial hypertension treated with first-line bosentan. *Eur J Clin Invest*. 2006 Sep;36 Suppl 3:10-5.
72. Clozel M, Breu V, Gray GA, *et al*. 1994. Phamacological characterization of bosentan, a new potent orally active nonpeptideendothelin receptor antagonist. *J Pharmacol Exp Ther*, 270:228–35.
73. Rich S, McLaughlin VV, 2003. Endothelin receptor blockers in cardiovascular disease. Circulation, 108:2184–90.
74. Liu C, Chen J. 2006. Endothelin receptor antagonists for pulmonary arterial hypertension. *Cochrane Database Syst Rev*, 3, CD004434.
75. Pollack, Andrew (2007-06-16). "Gilead'sDruIS Approved to Treat a Rare Disease". *New York Times*.Retrieved 2007-05-25.
76. "U.S. Food and Drug Administration Approves Gilead's Letairis Treatment of Pulmonary Arterial Hypertension" (Press release). *Gilead Sciences*. 2007-06-15. Retrieved 2007-06-16
77. "FDA Approves New Orphan Drug for Treatment of Pulmonary Arterial Hypertension" (Press release). *Food and Drug Administration*. 2007-06-15. Retrieved 2007-06-22.
78. "GlaxoSmithKline's Volibris (ambrisentan) receives authorisation from the European Commission for the treatment of Functional Class II and III Pulmonary Arterial Hypertension" (Press release).*GlaxoSmithKline*. 2008-04-25. Retrieved 2008-04-29.
79. Riechers H, Albrecht HP, Amberg W, *et al*. Discovery and optimization of a novel class of orally active nonpeptidicendothelin-A receptor antagonists. *J Med Chem* 1996;39:2123–2128.
80. Boss C, Bolli M, Weller T: Endothelin receptor antagonists: Structures, synthesis, selectivity and therapeutic applications. *Curr Med Chem* 2002;9:349–383.
81. Letairis.com. How to get Letairis.http://www.letairis.com/how_to_get_letairis. html (accessed 2007 August 1).
82. Food and Drug Administration. FDA labelling information [online], <http://www.fda.gov/cder/foi/ label/2007/022081s000lbl.pdf> (2007).
83. Bolli MH, Marfurt J, Grisostomi C *et al*. Novel benzo[1,4]diazepin-2-one derivatives as endothelin receptor antagonists. *J Med Chem* 2004; 47:2776–2795.

84. Greene S, Nunley K, Weber S *et al*. ETA vs. ETB receptor selectivity of endothelin-1 receptor antagonists. *J Am CollCardiol* 2006; 47:307A.
85. Barst RJ. Sitaxsentan: a selective endothelin-A receptor antagonist, for the treatment of pulmonary arterial hypertension. *Expert Opin Pharmacother* 2007; 8:95–109.
86. Gilead U. Letairis® (Ambrisentan) Full Prescribing Information. 2008.
87. Eriksson C, Gustavsson A, Kronvall T, Tysk C. Hepatotoxicity by bosentan in a patient with portopulmonary hypertension: a case-report and review of the literature. *J Gastrointestin Liver Dis* 2011;20(1):77–80.
88. McGoon MD, Frost AE, Oudiz RJ, Badesch DB, Galie N, Olschewski H, *et al*. Ambrisentan therapy in patientswith pulmonary arterial hypertension who discontinued bosentan or sitaxsentan due to liver function test abnormalities. Chest 2009;135(1):1229.
89. Letairis [package insert]. Foster City, CA: *Gilead Sciences*, Inc; 2007.
90. Riechers, H. *et al*. Discovery and optimization of a novel class of orally active non peptidicendothelin-A receptor antagonists. *J. Med. Chem*. 39, 2123–2128 (1996).
91. Rubin, L. J. *et al*. Bosentan therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* 346, 896–903 (2002).
92. Billman,G. E. (2002). Ambrisentan (Myogen). *Curr Opin Investig Drugs* 3,1483– 1486.
93. Amberg W, Hergenroder S, Hillen H, *et al*. 1999. LU208075 and LU302146, two novel ETA-selective endothelin receptor antagonists: SAR of 3,3-diaryl propionic acid derivatives. *International Conference on Endothelin*, 6 October 10–13P.
94. FDA/CDER Letairis (ambrisentan tablets) Clinical Pharmacology. *Biopharmaceutics* Review 3:98–99.Accessed May 28 2008. URL:http://www.fda.gov/cder/foi/nda/2007/022081s000_ClinPharmR_P3.pdf .
95. Opitz, CF, Ewert R, Kirch W, *et al*. 2008. Inhibition of endothelin receptors in the treatment of pulmonary arterial hypertension: does selectivity matter? Eur Heart J.
96. Letairis package insert. Foster City. CA: *Gilead Sciences* Inc.; 2007 June.
97. Tracleer package insert. South San Francisco. DA: Actelion Pharmaceuticals US, Inc; 2007 April.
98. Rubin LJ, Dufton C, Gerber MJ. 2005b. Ambrisentan for pulmonary arterial hypertension. *Future Cardiol*, 1:1–8.
99. Oudiz R, Torres F, Frost A, *et al*. 2006. ARIES-1: A placebo-controlled efficacy and safety study of ambrisentan in patients with pulmonary arterial hypertension. *Chest*, 130:121S.
100. Letairis (ambrisentan) tablets for oral use. Full prescribing information.http://www.letairis.com/downloads/LETAIRIS_prescribing_information.pdf .Accessed November 13, 2007.
101. Kenyon KW, Nappi JM. 2003. Bosentan for the treatment of pulmonary arterial hypertension. *Ann Pharmacother*, 37:1055–62.
102. Barst RJ, Langleben D, Frost A, *et al*. 2004a. Sitaxsentan therapy for pulmonary arterial hypertension. *Am J RespirCrit Care Med*, 169:441–7.
103. Gerber MJ, Dufton C, Pentikis H, *et al*. Ambrisentan has no clinically relevant effect on the pharmacokinetics or pharmacodynamics of warfarin. *Chest*. 2006;130(Suppl 4): 256S. Abstract.
104. Dufton C, Gerber M, Yin O, *et al*. No clinically relevant pharmacokinetic interaction between ambrisentan and sildenafil. *Chest*. 2006;130(Suppl 4):254S. Abstract.
105. Galie N, Badesch D, Oudiz R, *et al*. 2005a. Ambrisentan therapy for pulmonaryarterial hypertension. *J Am CollCardiol*, 46:529–35.
106. Olschewski H, Galie N, Ghofrani HA, *et al*. 2006. Ambrisentan improves exercise capacity and time to clinical worsening in patients with pulmonary arterial hypertension: Results of the ARIES-2 study. *Proc Am ThoracSoc*, 3:A728.
107. Letairis (ambrisentan) tablets for oral use. Full prescribing information. http://www.letairis.com/downloads/LETAIR

IS_ prescribing_information.pdf. Accessed November 13, 2007.
108. Galie N, Keogh AM, Frost A, *et al*. 2005c. Ambrisentan long-term safety and efficacy in pulmonary arterial hypertension-one year follow up. *Proc Am ThoracSoc*, 2:A299.
109. Clozel M. Effects of bosentan on cellular processes involved in pulmonary arterial hypertension: Do they explain the long-term benefit? *Ann Med* 2003;35:605–613.
110. Lee SH, Rubin LJ. Current treatment strategies for pulmonary arterial hypertension. *J Intern Med* 2005;258: 199–215.
111. Mylona P, Cleland JG. Update of REACH-1 and MERIT-HF clinical trials in heart failure. Cardio.net Editorial Team. *Eur J Heart Fail* 1999;1:197–200.
112. Galie N, Manes A, Uguccioni L, *et al*. Primary pulmonary hypertension: insights into pathogenesis from epidemiology. *Chest.* 1998;114(suppl):184S-94S.
113. Hoeper MM, Markevych I, Spiekerkoetter E, Welte T, Niedermeyer J. Goal-oriented treatment and combination therapy for pulmonary arterial hypertension. *Eur Respir J.* 2005;26:858-863.
114. McLaughlin VV, Oudiz RJ, Frost A, *et al*. Randomized study of adding inhaled iloprost to existing bosentan in pulmonary arterial hypertension. *Am J RespirCrit Care Med.* 2006;174:1257-1263.
115. Gabel G, Uhlmann D, Teupser D, *et al*. Influence of a selective endothelin(A) receptor antagonist on the quantitative mRNA expression and the immunohistochemistry of vasoactive mediators after pancreas transplantation. *Transplant Proc* 2003;35:2137–2138.
116. Martignoni ME, Ceyhan GO, Ayuni E, *et al*. Endothelin receptor antagonists are not beneficial in the therapy of acute experimental pancreatitis. *Langenbeck's Arch Surg* 2004;389:184–192.
117. Uhlmann D, Gabel G, Ludwig S, *et al*. Effects of ET(A) receptor antagonism on proinflammatory gene expression and microcirculation following hepatic ischemiareperfusion. *Microcirculation* 2005;12:405–419.
118. Uhlmann D, Gaebel G, Armann B, *et al*. Attenuation of proinflammatory gene expression and microcirculatory disturbances by endothelin A receptor blockade after orthotopic liver transplantation in pigs. *Surgery* 2006;139:61–72.
119. Witzigmann H, Ludwig S, Escher E, *et al*. Administration of a selective endothelin-A receptor antagonist (BSF 208075) improves hepatic warm ischemiareperfusion injury in pigs. *Transplant Proc* 2002;34: 2387–2388.
120. Kirchengast M. Endothelin receptor blockade and in-stent restenosis. *J Cardiovasc Pharmacol* 2001; 38(Suppl 2):S31–S34.
121. Uhlmann D, Glasser S, Gaebel G, *et al*. Improvement of postischemic hepatic microcirculation after endothelin A receptor blockade — endothelin antagonism influences platelet-endothelium interactions. *J Gastrointest Surg* 2005;9:187–197.

Table 1. Current medications approved for the treatment of pulmonary arterial hypertension

Generic name	Trade name	Drug class	Administration routes	Data approved
Epoprostenol	Flolan	Prostacyclin analogue	Continous IV infusion	1995
Treprostnil	Remodulin	Prostacyclin analogue	Continous IV or SQ infusion	2002 (SQ) 2004(IV)
Lloprost	Ventavis	Prostacyclin analogue	Nebulized inhalation 6-9 times daily	2004
Bosentan	Tracleer	Non-selective endothelin Receptor antagonist	Oral twice daily	2001
Ambrisentan	Letairis	Selective endothelin Receptor antagonist	Oral once daily	2007
Sitaxsentan	Thelin	Non-selective endothelin Receptor antagonist	Oral once daily	2006 (Europe, Canada)
Sildenafil	Revatio	Phosphodiesterase Type 5 inhibitor	Oral thrice daily	2005
Beraprost		Prostacyclin analogue	Oral 1 to 3 times per day	1994 (Japan)

Table 2. Pulmonary Arterial Hypertension (PAH) Classification and Select Modifications to 2003 WHO Clinical Classification of PH

Updated 2008 World Health Organization (WHO) Clinical Classification of Pulmonary Hypertension (PH)
1. PAH • Idiopatic PAH • Heritable BMPR2, ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia), unknown • Drug- and toxin- induced • Associated with connective tissue diseases, HIV, portal hypertension, congenital heart disease, schistosomiasis, chronic hemolytic anemia • Persistent PH of the newborn • Pulmonary veno-occlusive disease and/or pulmonary capilaryhemangiomatosis
2. PH owing to left heart disease
3. PH owing to lung diseases and/or hypoxia • Other pulmonary disease with mixed restrictive and obstructive pattern
4. Chronic thromboembolic pulmonary hypertension
5. PH with unclear multifactorial mechanisms • Hematologic disorders: splenectomy, myeloprohferative disorders • Systemic disorders: sarcoidosis, vasculitis, neurofibromatosis • Metabolic disorders: thyroid disorders • Others: chronic renal failure on dialysis

Table 3. Use of ambrisentan in animal models and clinical trials

PATHOLOGY	MODEL	REFERENCE
Animal Models		
Ischemia reperfusion pancreas	Pig	115
Ischemia reperfusion pancreas	Rat	116
Ischemia reperfusion liver	Pig	117,118,119
Ischemia reperfusion liver	Rat	120
Angioplastyrestenosis	Pig	121
Clinical Trials		
Cardiovascular disease	Phase I	*not available (92)
Renal failure	Phase I	
Renal failure	Phase II	
Hypertension	Phase II	
Cardiac failure	Phase II	
Cardiovascular disease	Phase II	
PAH	Phase II	105
PAH (ARIES-1)	Phase III	**Myogen(no data available)
PAH (ARIES-2)	Phase III	**Myogen (data available)

*Clinical trials, mentioned in the review by Billman *et al*. (92), no further details available.
**Clinical trials, reported on the Myogen homepage, but not yet published.

Table 4. Changes from Baseline in 6-Minute Walk Distance (meters)

	ARIES-1			ARIES-2		
	Placebo (N=67)	5mg (N=67)	10mg (N=67)	Placebo (N=65)	2.5mg (N=64)	5mg (N=63)
Baseline	342±73	340±77	342±78	343±86	347±84	355±84
Mean change from Baseline	-8±79	23±83	44±63	-10±94	22±83	49±75
Placebo-adjusted mean change from baseline	-	31	51	-	32	59
Placebo-adjusted mean change from baseline	-	27	39	-	30	45
p-value[a]	-	0.008	<0.001	-	0.022	<0.001

Mean ± standard deviation

a. p-values are Wilcoxon rank sum test comparisons of LETAIRIS to placebo at Week 12 stratified by idiopathic or heritable PAH and non-idiopathic, non-heritable PAH patients.

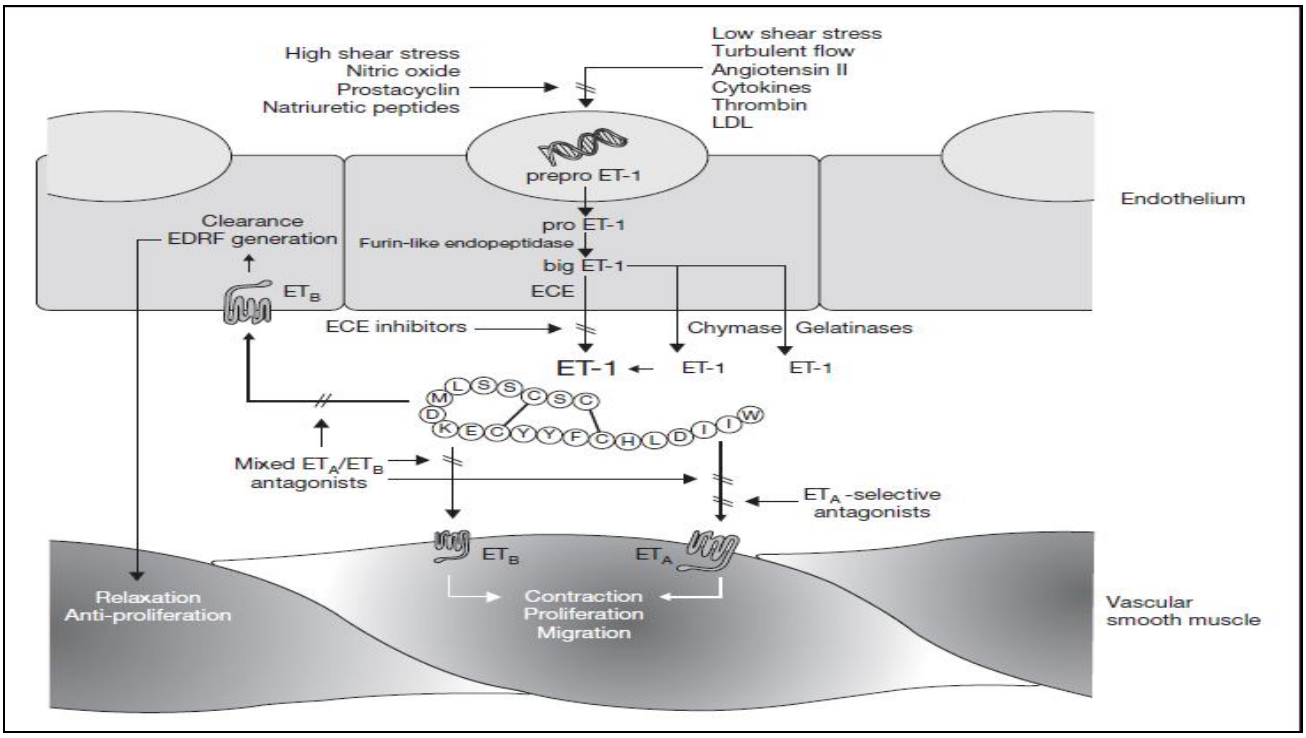


Figure.1. Schematic illustration of the endothelin system (redrawn with permission from http://www.nature.com/bjp/journal/v153/n6/fig_tab/0707516f1.html).ECE=endothelinconverting enzyme; EDRF=endothelium-derived relaxing factor; ET = endothelin; LDL = low-density lipoprotein.

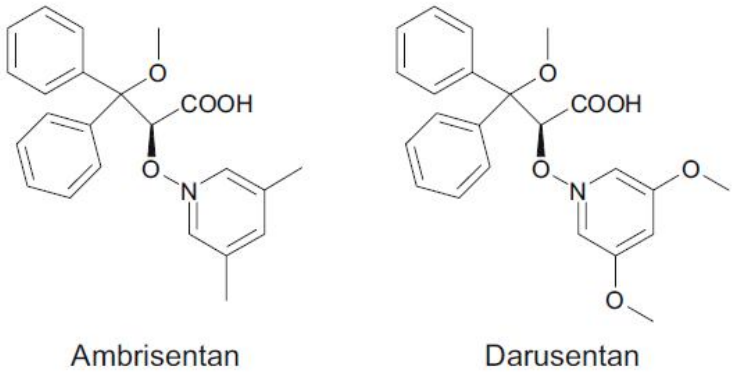


Figure.2. Chemical scaffold of ambrisentan (LU 208075) ((+)-(S)-2-(4,6-dimethyl pyrimidin-2-yloxy)-3-methoxy- 3,3-diphenyl-propionic acid) and the structurally similar darusentan (LU 1352521) ((+)-(S)-2(4,6-dimethoxy- pyrimidin-2-xyloxy)-3-methoxy-3,3-diphenyl-propionic acid).

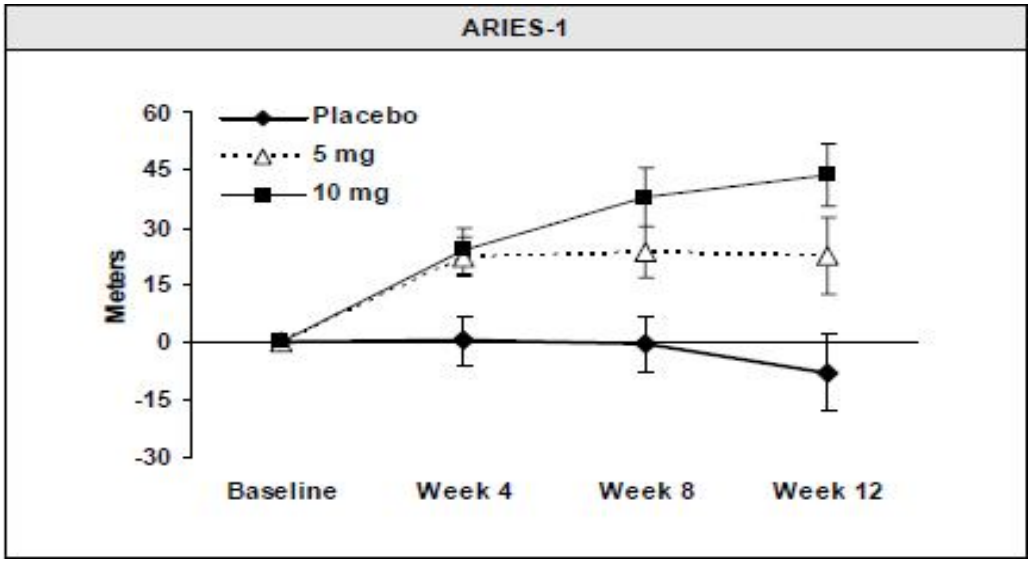


Figure.3. Mean Change in 6-Minute Walk Distance

Mean change from baseline in 6-minute walk distance in the placebo and LETAIRIS groups Values are expressed as mean ± standard error of the mean.