ENDOTHELIN RECEPTOR ANTAGONISTS — THEIR ROLE IN PULMONARY MEDICINE

S. Boniface, M. Reynaud-Gaubert

Introduction.

Understanding of the function of endothelin-1 in the pathophysiology of endothelial disease, in particular pulmonary arterial hypertension (PAH), has paved the way for the development of endothelin-receptor antagonists (ERAs) and explains the leading role they now play in the treatment armamentarium for this disease.

Background.

Three active ERA drugs (bosentan, sitaxentan, ambrisentan) are currently approved for the treatment of PAH in France. Several randomised clinical trials have demonstrated their efficacy and safety in PAH.

Perspectives and conclusion.

Besides its vasoconstrictor effect, endothelin-1 (ET-1) plays a pivotal role in cell proliferation and apoptosis. ERAs are innovative drugs potentially useful in some pulmonary disorders such as idiopathic pulmonary fibrosis or systemic sclerosis, even though the preliminary results published remain insufficient or controversial.

Conclusion.

ERAs play a major role in the management of pulmonary vascular disease. Other drugs, still under study, could prove useful in the treatment of infiltrating pneumonias.

Introduction

Endothelin-1 (ET-1) is a peptide produced by endothelial cells discovered in 1988 by Yanagisawa et al. [1]. The identification of its vasomodulator properties and its role in pulmonary...
vascular remodeling have made ET-1 a major mediator in the pathophysiology of pulmonary arterial hypertension (PAH) [2], and ET-1 receptor antagonists (ERAs) have become essential drugs in the treatment armamentarium for PAH [3,4]. Since marketing of the first ERA (bosentan), other antagonists have been developed and approved for this indication [5]. Besides its vasomodulator action, ET-1 also plays a role in the differentiation process, cell proliferation and apoptosis [6], providing ERAs with therapeutic potential in other diseases.

In this review, we first discuss the biological properties and mechanisms of action of endothelin and ERAs. We then report the main results obtained with ERAs in PAH, as well as therapeutic perspectives in other lung diseases, primarily pulmonary fibrosis.

The endothelial system

Endothelin-1 (ET-1) is a 21-amino acid peptide containing with two intramolecular disulfide bonds [1]. The production of active ET is genetically controlled, and results from the cleavage of preproendothelin, an inactive precursor, generating big-ET, under the effect of a metalloproteinase: endothelin-converting enzyme [6]. There are three ET isoforms, ET-1, -2 and -3, similar in structure but derived from a distinct gene. ET-1 is the most widely distributed in the body, and the most involved in PAH. These endothelins are mainly produced by endothelial cells, but also by other cell components (epithelial or smooth muscle cells, cardiomyocytes, fibroblasts, macrophages, and keratinocytes) [7]. Under physiological conditions, hypoxia results in the production and release of ET primarily at the basal pole of endothelial cells. This production of ET is directed mainly to tissues with low circulatory volume, hence its role for the most part in the autocrine-paracrine mechanism [8]. The metabolism of ET is still incompletely known, but the lung seems to be the main production site. Tissue production of ET is increased in certain diseases such as PAH, where an elevated plasma ET-1 level has been demonstrated [9]. Endothelins exert their action via stimulation of two receptors, types A and B, called ETA and ETB (Fig. 1) [7]. ET-1 has an affinity for ETA 100 times greater than other isoforms, while ETB affinity is comparable between the three isoforms. ET receptors belong to the G protein-coupled superfamily, responsible for the activation of phospholipase C, increasing intracellular calcium and activation of protein kinase C (Fig. 2) [7]. They also promote the release of arachidonic acid via activation of phospholipase A2 and increase the mitogenic activity of certain cell types such as smooth muscle cells and fibroblasts [7].

In the pulmonary arteries, ETA and ETB are expressed by smooth muscle cells, but only ETB is present in endothelial cells [12]. Moreover, ETB expression is greater than that of ETA in the distal pulmonary arteries [13]. Thus simultaneous activation of ETA and ETB causes vasoconstriction mediated by the proliferation of smooth muscle cells and fibroblasts that participate in vascular remodeling [13], whereas the isolated activation of ETB results in vasodilation via the release of substances such as nitric oxide and prostacyclin [14]. ETB also participates in the regulation of endothelial apoptosis by limiting it and increasing plasma ET-1 clearance [7,15]. Finally, it inhibits the ET converting enzyme required for production of mature ET-1 [6].

There is probably an interaction between ETA and ETB receptors, which then behave as a heterodimer regulating the vasomodulator effect of ET-1 [13,16]. Conversely, high levels of ET-1 have a negative impact on receptor expression, particularly of ETA [16]. In some diseases such as systemic sclerosis, the superiority of the constrictive and cell proliferation effect of ETA in vitro compared to that of ETB [17], is not regularly found in vivo [18]. Finally, in patients with PAH, ETB expression and functionality is apparently increased in smooth muscle cells, but these findings remain controversial [13,19]. Thus the respective actions of ET receptors and signaling pathways between each other and with their ligands are complex and the pathophysiological consequences are still poorly defined.

ERA selectivity is different for ETA and ETB. Bosentan is a mixed antagonist of ETA and ETB, while sitaxentan and ambrisentan, called “selective antagonists”, show predominant anti-ETA action in vitro. The potential impact of this anti-ETA selectivity is yet to be demonstrated in clinical practice [4,20,21].

ET-1 receptor antagonist (ERA) in pulmonary arterial hypertension (PAH)

PAH is a rare and severe disease, characterized by progressive remodeling of the pulmonary arteries associated with proliferation of fibrous tissue in the intima and media of the vessel walls. This reduces the diameter of the arterial lumen, sometimes worsened by an obstructing clot, resulting in persistent elevation in pulmonary artery resistances and pressures. PAH is contributed to and maintained by endothelial dysfunction associated with smooth muscle abnormalities in the context of genetic predisposition and/or environmental factors that are not always identified [2,22].
Endothelin-I is synthesized mainly by endothelial cells under the effect of hypoxia.

- It is synthesized mainly in the lung tissue and its production increases in PAH.
- Endothelins act through stimulation of two types of receptors, A and B, called ETA and ETB.
- The simultaneous activation of ETA and ETB causes vasoconstriction mediated by the proliferation of smooth muscle cells and fibroblasts, whereas the isolated activation of ETB results in vasodilation via the release of substances such as nitric oxide and prostacyclin.
- There is interaction between ETA and ETB receptors, which then behave as a heterodimer regulating the vasomodulator effect of ET-1.

PAH is defined as an elevated mean pulmonary artery pressure (PAPm) of over 25 mmHg at rest with a pulmonary artery occlusion pressure below 15 mmHg [2]. The classification of PAH based on etiology currently used is the Dana Point classification recently published [23]. Group I in the Venice classification, precapillary pulmonary hypertension, included five diseases: idiopathic PAH; familial PAH; PAH associated with various disorders such as connective tissue disease, congenital systemic-to-pulmonary shunts, portal hypertension, HIV infection, toxic or anorectic drugs; and more recently veno-occlusive disease and pulmonary capillary hemangiomatosis.

Before the introduction of prostacyclin in 1996, median survival was less than 2 years from diagnosis [24]. Even though over 10 years later, prostacyclin remains the standard treatment in advanced disease, the considerable constraints imposed by continuous intravenous administration and the serious side effects have paved the way for the development of new drugs and new formulations. Prostacyclin analogs have thus emerged, such as inhaled iloprost and subcutaneous treprostinil; three ERAs are active when taken orally. However, the specific adverse effects of each drug, and follow-up that remains insufficient, make them a last resort indication for the time being [22]. The clinical benefit and ease of oral administration of ERAs, have rapidly established these drugs in the treatment armamentarium for PAH. The range of oral drugs was then expanded with the introduction of phosphodiesterase type 5 inhibitors (sildenafil citrate, tadalafil), with a different but complementary mechanism of action to previous drugs, thus providing treatment alternatives and/or associations when single-drug therapy failed [2]. Current treatment guidelines are based on the evidence-based treatment algorithm using the NYHA function classification for PAH [5].

Three oral ERAs are currently available in France. The volume of publications and the length of studies are related respectively to their date of availability.

**Bosentan**

Bosentan (Tracleer®, Actelion), the first ERA to be marketed, has been the subject of numerous publications since the reference study [25]. In 2001, it was approved by the FDA in the United States and Canada for the treatment of NYHA functional class III and IV PAH. It was available in France in 2001, with a temporary authorization, until a marketing authorization (AMM) was granted in 2002 for treatment of NYHA functional class III PAH [26]. Extension of the AMM was obtained in 2007 for PAH associated with congenital...
• PAH is a rare and severe disease, characterized by proliferation of fibrous tissue in the intima and media of the vessel walls. This reduces the diameter of the arterial lumen, sometimes worsened by an obstructing clot, resulting in persistent elevation in pulmonary artery resistances and pressures.

• Prostacyclin remains the standard treatment in advanced disease, but the considerable constraints imposed by continuous intravenous administration and the serious side effects have paved the way for the development of new drugs and new formulations.

heart disease, and in 2008 for prevention of digital ulcers in systemic sclerosis, regardless of the presence of PAH.

Pharmacological data
Bosentan is a highly specific ET-1 antagonist. Even though it has a higher affinity for ETA receptors, it is considered to be a mixed ET-1 receptor antagonist.

Pharmacokinetics
Treatment is started at the dose of 62.5 mg twice a day for four weeks; it is then increased until the maintenance dose of 125 mg twice a day is reached. It has a half-life of approximately 7 hours and an oral bioavailability of 50% [27]. Considering the potential hepatotoxicity attributed to this therapeutic classification, and the dose-dependent toxicity of this drug, monthly monitoring of transaminase levels is advised. There are no contraindications to its use, or dose adjustments required, in mild liver disease (Child-Pugh grade A), but bosentan is not recommended in more severe liver impairment [28]. Dose adjustment is not required in impaired renal function [29].

Drug interactions
The metabolism of bosentan is mediated by the cytochrome enzyme P450 (CP450), and in particular by its isoenzymes CYP2C9 and 3A4, thus explaining the potential interactions with certain enzyme inducers such as oral anticoagulants. In principle, the co-administration of bosentan and warfarin does not require treatment adjustment, even though closer surveillance of INR is advised [25,26,30]. The results are similar with digoxin [31].

Because of the considerable risk of worsening PAH in pregnancy, effective contraception is strongly recommended [32]. Concerning bosentan, oral hormonal contraceptives are contraindicated due to their decreased efficacy and the risk of teratogenicity [33].

Clinical data
Because of the large number of publications, only randomised controlled studies will be discussed. The main studies are summarized in Table 1.

Efficacy and tolerance
The first published clinical study was a Phase II, multicenter, randomised, placebo-controlled study involving 32 patients with idiopathic PAH (n = 27), or PAH associated with systemic sclerosis (n = 5) [25], NYHA functional class III on inclusion, and treated for 12 weeks. The primary endpoint was exercise tolerance assessed using the 6-minute walk distance (6MWD) whose mean value on inclusion was 355 m (± 82) and 390 m (± 86) respectively for the placebo group and the treatment group. The secondary endpoints were haemodynamic (cardiac index [CI]; pulmonary vascular resistances [PVR]; mean pulmonary artery pressure [mPAP]; right atrial pressure [RAP]) and functional (Borg dyspnoea scale, NYHA stage). The mean values for these parameters on inclusion were respectively, for the placebo group and the treatment group: 2.5 (± 1.0) and 2.4 (± 0.7) L min⁻¹m⁻² for CI, 942 (± 430) and 896 (± 425) dyne s cm⁻⁵ for PVR, and 56 (± 10) and 54 (± 13) mmHg for PAP. At the end of the study, all criteria had improved in the bosentan group: a gain of 70 m in the 6MWD from the 8th week, an increase in CI of 1 L min⁻¹m⁻², and a decrease in PVR of 223 dyne s cm⁻⁵. Concerning NYHA stage, 49% of the patients treated with bosentan progressed from NYHA functional class III to II, 51% remained stage III, and none developed functional class IV. Extension to an open-label study for 28 weeks showed maintenance of clinical and functional benefit at 12-month follow-up, while the haemodynamic improvement was only maintained for PVR [41].

The following phase III placebo-controlled study (BREATHE-1, Bosentan Randomised trial of Endothelin Antagonist THERapy) assessed the efficacy of bosentan (two arms, 125 and 250 mg) versus placebo in 213 patients with idiopathic PAH (70%) or PAH associated with connective tissue disease for 16 weeks [26]. The endpoints were comparable to those of the previous study, but there were no haemodynamic tests at 16 weeks. There was a significant improvement in all criteria assessed in both treatment groups. Liver toxicity, found in 9% of cases, was dose-dependent with a significant prevalence in the 250 mg group versus the 125 mg group, requiring interruption of treatment in three cases. A multicenter post-marketing authorization surveillance programme run by the European Medicines Agency for 30 months that included 5000 patients, i.e. 79% of patients treated in Europe, showed an annual incidence of increased transaminase levels of 10.1%, with interruption of bosentan being required in 3.2% of cases [40]. Subsequent studies have confirmed this incidence.

Long-term impact
An open-label extension study of two previous trials [25,26] was used to assess survival under treatment in 169 patients over a period of up to three years [42]. Specific additional treatment of PAH was authorized depending on clinical status as assessed by the investigator. Only 39 patients required recourse to additional treatment with bosentan. NYHA functional class IV and a 6MWD below 358 m were factors indicating poor survival. These results were comparable with those of other studies [43,44].

Early treatment of pulmonary arterial hypertension (PAH)
The studies that led to the registration of bosentan mainly concerned a population with PAH in NYHA functional class III, until the recently published the Endothelin Antagonist tRrial in miLDY symptomatic PAH patients (EARLY) trial [38]. This multicenter, randomised, double-blind, placebo-controlled trial involved 185 patients in NYHA functional class II, treated for 6 months. The primary endpoints were 6MWD (434 m on average at inclusion) and PVR. At 6 months of treatment, the benefit with bosentan was + 19 m in 6MWD,
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>No. patients</th>
<th>Treatment</th>
<th>Initial NYHA stage</th>
<th>Primary endpoint results</th>
<th>Results Haemodynamic</th>
<th>Other criteria</th>
<th>Tolerance Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channick 2001 [25]</td>
<td>R, DB, PC 12 W</td>
<td>32 adults</td>
<td>PAHi, c</td>
<td>62.5 mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 125 mg × 2/day or 250 mg × 2/day</td>
<td>III (100%)</td>
<td>+ 70 m on 6MWD</td>
<td>↑ CI ↓ PVR ↓ RAP, mPAP</td>
<td>NYHA Class II (47%) Class IV (0%)</td>
</tr>
<tr>
<td>Rubin 2002 [26] BREATHE-1</td>
<td>R, DB, PC 16 W</td>
<td>213 adults PAHi, c</td>
<td>62.5 mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 125 mg × 2/day or 250 mg × 2/day</td>
<td>III (86.3%) IV (7.5%)</td>
<td>+ 44 m on 6MWD</td>
<td>NP</td>
<td>NYHA Class II (34%) Class I (3%)</td>
<td>↑ transaminases (9%) dose dependent</td>
</tr>
<tr>
<td>Humbert 2004 [34] BREATHE-2</td>
<td>R, DB, PC 16 W</td>
<td>33 adults PAHi, c</td>
<td>250 mg × 2/day Epoprostenol 62.5 mg × 2/day then 125 mg × 2/day 31.2 or 62.5 or 125 mg × 1/day 4 weeks then 2/day</td>
<td>III (75.7%) IV (24.3%)</td>
<td>−36.3 ± 4 PVR (ns)</td>
<td>Improvement in all parameters (ns)</td>
<td>NYHA Class I/II (48%) Class III (46%) Class IV (6%)</td>
<td>↑ Transaminases (18%)</td>
</tr>
<tr>
<td>Barst 2003 [35] BREATHE-3</td>
<td>OS, NC 12 W</td>
<td>19 children PAHi, c</td>
<td>Epoprostenol 62.5 mg × 2/day then 125 mg × 2/day 31.2 or 62.5 or 125 mg × 1/day 4 weeks then 2/day</td>
<td>II (79%) III (21%)</td>
<td>−300 dynes.s.cm⁻⁵ PVR − 8 mmHg PAP</td>
<td>Improvement in NYHA (26%)</td>
<td>↑ Transaminases (15%)</td>
<td></td>
</tr>
<tr>
<td>Sitbon 2004 [36] BREATHE-4</td>
<td>OS, NC 16 W</td>
<td>16 HIV+</td>
<td>62.5 mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 2/day</td>
<td>III (94%) IV (6%)</td>
<td>+ 91 m on 6MWD</td>
<td>↑ CI ↓ PVR</td>
<td>NYHA Class I/II (81%) Class III (19%)</td>
<td>↑ Transient transaminases (12%) ↑ QoL</td>
</tr>
<tr>
<td>Galiè 2006 [37] BREATHE-5</td>
<td>R, DB, PC 16 W</td>
<td>54 Eisenmenger</td>
<td>62.5 mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 2/day</td>
<td>III (100%)</td>
<td>+ 1% SaO₂ −472 dynes.s.cm⁻⁵ PVR</td>
<td>↓ mPAP</td>
<td>+ 53 m 6MWD</td>
<td>NYHA Class II (35%) Class III (64%) Class IV (3%) Improvement in time to worsening ↑ Transaminases (3%)</td>
</tr>
<tr>
<td>Rubin 2008 [38] EARLY</td>
<td>R, DB, PC 6 months</td>
<td>185</td>
<td>62.5mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 125 mg × 2/day</td>
<td>II (100%)</td>
<td>− 22.6% PVR + 19 m 6MWD (ns)</td>
<td>Improvement in all parameters Improvement in time to worsening</td>
<td>↑ Transaminases (13%)</td>
<td></td>
</tr>
<tr>
<td>Jais 2008 [39] BENEFIT</td>
<td>R, DB, PC 16 W</td>
<td>157 PE PH</td>
<td>62.5mg × 2/day 4 weeks then 125 mg × 2/day 4 weeks then 125 mg × 2/day</td>
<td>II (28%) III (68%) IV (3%)</td>
<td>↓ PVR 6MWD (ns)</td>
<td>Improvement in all parameters Improvement of NYHA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

R: randomised; DB: double-blind; PC: placebo-controlled; OS: open study; NC: not controlled; NA: not available; W: weeks; 6MWD: 6-minute walk distance; NP: not performed; (ns): not statistically significant; PVR: pulmonary vascular resistance; CI: cardiac index; RAP: right atrial pressure; mPAP: mean pulmonary artery pressure; PAHi, c: PAH, idiopathic or with connective tissue disease; PE PH: Post-embolic pulmonary hypertension; QoL: quality of life; SaO₂: arterial oxygen saturation.
Endothelin receptor antagonists in pulmonary medicine

Therapeutic indications in non-idiopathic pulmonary arterial hypertension (PAH)

Most clinical trials have included indiscriminately patients with precapillary idiopathic PAH, or those with PAH associated with connective tissue disease or another disorder, sometimes making results difficult to interpret because of the underlying pathogenesis. Thus, when the cohort allowed for it, analysis of sub-groups has provided interesting data. Specific analysis of the population of patients with connective tissue disease initially included in the pivotal clinical trials cited above [25,26], whether systemic sclerosis (n = 52), systemic lupus erythematosus (n = 8) or other mixed connective tissue diseases (n = 6), shows that the benefit of bosentan in this context is less than in idiopathic PAH [46]. This emphasizes the spontaneously poorer prognosis of PAH associated with systemic sclerosis compared with idiopathic PAH, with survival rates of 85.9% and 73.4% respectively at 1 and 2 years [46]. The survival rate at 2 years for PAH associated with systemic sclerosis before the introduction of ERAs was below 60% [47,48]. In the TRUST (TRacleer Use in PAH associated with Scleroderma and Connective Tissue Diseases) trial, 53 patients with PAH in the context of connective tissue disease received open-label bosentan 125 mg twice a day for 48 weeks. At the end of the study, 27% of the patients showed an improvement in NYHA functional class, survival rate was estimated at 92%, and absence of clinical worsening at 68% [49]. The efficacy of bosentan is also recognized in Eisenmenger syndrome [37], in HIV-associated PAH [36], and PAH in children [35] (Table 1). In portopulmonary hypertension, Hoep et al. showed haemodynamic and functional effectiveness of bosentan in Child-Pugh class A patients with good liver tolerance with more than a year of treatment [50].

The utility of the drug has also been noted in postembolic pulmonary hypertension (PEPH), not associated with thromboendarterectomy [44,51]. The BENEFIT study (Bosentan Effects in InNOpErable Forms of chronic Thromboembolic pulmonary hypertension), a randomised placebo-controlled trial involving 157 patients with inoperable chronic thromboembolic pulmonary hypertension (CTEPH) or persistent CTEPH after surgery, showed improved haemodynamic parameters at 16 weeks, but little change in 6MWD in both groups [39]. These results could be explained by the considerable physical deconditioning in this population.

Finally, in PAH associated with chronic respiratory failure, there are currently no published randomized controlled trials [52].

Combination therapy

Combination therapies using different pharmacological classes are currently widely prescribed despite a low level of proof in terms of benefit of these associations. The BREATHE-2 trial, which evaluated the association of bosentan with epoprostenol versus epoprostenol alone, did not show significant results, possibly because of the small cohort [34]. The combination of inhaled iloprost and bosentan used in two randomised controlled trials, The Safety and Pilot Efficacy Trial in Combination with Bosentan for Evaluation in Pulmonary Arterial Hypertension (STEP-1) [53] and Combination therapy of Bosentan and aerosolised iloprost in Idiopathic Pulmonary Arterial Hypertension (COMBI) [54], only showed clinical improvement in the STEP-1 trial, with satisfactory tolerance in both trials. The COMPASS studies (Effects of Combination of Bosentan and Sildenafil vs. Sildenafil Monotherapy on Morbidity and Mortality in Symptomatic Patients With Pulmonary Arterial Hypertension) evaluated the association of bosentan with sildenafil [55,56]. As they have in common liver metabolism mediated by CP450, the supposed pharmacokinetic interaction between the two drugs has been confirmed; this results in a reduction in the bioavailability of sildenafil and an increase in that of bosentan [57]. Results from the open COMPASS-1 study report similar haemodynamic efficacy between the groups sildenafil alone and sildenafil plus bosentan [55]. The ongoing COMPASS-2 study uses a randomised and placebo-controlled protocol to evaluate the same combination therapy [56]. Clearly additional data are required to refine this combination therapy strategy.

- Bosentan, a highly specific ET-1 antagonist, is indicated in PAH and digital ulcers in systemic sclerosis.
- Several studies have shown its efficacy.
- Bosentan is more effective in idiopathic precapillary PAH than in PAH associated with connective tissue disease.
- It is also effective in Eisenmenger syndrome in HIV-associated pulmonary hypertension, pulmonary hypertension in children, portopulmonary hypertension, and postembolic pulmonary hypertension.
- The utility of combination therapy remains to be established.

Ambrisentan

Ambrisentan was approved in the United States in June 2007 by the FDA in the management of PAH in NYHA functional class II and III, and in Europe in April 2008 by the European Medicines Agency. In France, ambrisentan in single daily doses of 5 and 10 mg has been available since February 2009 (Volibris®, GlaxoSmithKline®).
Pharmacological data

Ambrisentan is a selective ET-1 antagonist with an affinity for ETA approximately 4000 times greater than that for ETB [58].

Pharmacokinetics

The half-life of ambrisentan in patients with PAH is approximately 15 hours, which explains the rationale behind a single daily dose, without regard to meals [58,59]. It is mainly metabolized in the liver by glucuronidation, but with relatively little effect on the CP450 isoenzymes [58,59]. Ambrisentan does not require dosage adjustment in mild to moderate renal failure. Liver tolerance of ambrisentan is satisfactory, but as no long-term follow-up studies are available, it is not recommended in severe liver failure.

Drug interactions

Co-administration of bosentan with sildenafil or warfarin does not require dose adjustment, even at the initial prescription [60,61]. Thus no specific surveillance of INR is required on introducing the treatment. The fact that ambrisentan is the only ERA with a propanoic acid structure may explain this lack of interaction with oral anticoagulants.

Clinical data

Seven trials have been conducted with ambrisentan in PAH, three of which have now been published [62,63]. The first was a phase II randomised, double-blind, dose ranging study, but without a placebo-treated group for 12 weeks [62]. Its objective was to evaluate the efficacy and safety of four doses of ambrisentan (1, 2.5, 5 or 10 mg daily) in 64 patients with idiopathic PAH (70%), PAH associated with connective tissue disease, anorectic drugs, or HIV infection. The primary endpoint was improvement in 6MWD (mean distance on inclusion of 343 ± 79 m); the secondary endpoints were functional and haemodynamic. At 12 weeks, the gain in 6MWD was +36 m (p < 0.001), whatever the dose. Improvement was also noted for other endpoints, in particular functional class (38% in class III against 64% initially, 50% in class II and 12% in class I), mPAP (−5.2 mmHg, p < 0.0001) and CI (+0.33 L/min/m², p < 0.0008), as well as time to clinical worsening. Clinical worsening was defined as the occurrence of death, hospitalization, changes in diuretic therapy, or the use of another specific treatment for PAH. The small cohort did not allow for the creation of subgroups with or without presentation of idiopathic PAH. Liver tolerance was satisfactory, not dose-dependent, with an incidence of 3%.

Ambrisentan has also been the subject of phase III randomised, double-blind, placebo-controlled, multicenter studies (ARIES-1 and ARIES-2: Ambrisentan in PAH-A Phase III, Randomised, Double-Blind, Placebo-Controlled, Multi-center Study of Efficacy in Subjects With Ambrisentan PAH) published together [63]. In the ARIES-1 study, 202 patients received 5 or 10 mg of ambrisentan once daily, while in ARIES-2, 192 patients received 2.5 or 5 mg versus placebo, the methodology being otherwise identical. The assessment criteria were practically superimposable on those of the phase II study, without haemodynamic evaluation, but

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical and haemodynamic patient data on inclusion in the ARIES-1 and ARIES-2 studies [63].</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARIES-1 n = 202</td>
</tr>
<tr>
<td>PAH diagnosis</td>
<td></td>
</tr>
<tr>
<td>Idiopathic PAH</td>
<td>63%</td>
</tr>
<tr>
<td>PAH associated with connective tissue disease</td>
<td>21%</td>
</tr>
<tr>
<td>PAH associated with anorexigen use</td>
<td>2.5%</td>
</tr>
<tr>
<td>PAH associated with HIV infection</td>
<td>3.5%</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.5%</td>
</tr>
<tr>
<td>II</td>
<td>32%</td>
</tr>
<tr>
<td>III</td>
<td>58%</td>
</tr>
<tr>
<td>IV</td>
<td>7%</td>
</tr>
<tr>
<td>Mean 6MWD (m)</td>
<td>341 ± 76</td>
</tr>
<tr>
<td>mPAP (mmHg)</td>
<td>47 ± 13 (5 mg ambrisentan)</td>
</tr>
<tr>
<td></td>
<td>51 ± 16 (10 mg ambrisentan)</td>
</tr>
<tr>
<td>Mean CI (L/min/m²)</td>
<td>2.5 ± 0.9 (5 mg ambrisentan)</td>
</tr>
<tr>
<td></td>
<td>2.6 ± 0.7 (10 mg ambrisentan)</td>
</tr>
<tr>
<td>Mean PVR (dynes/s/cm⁵)</td>
<td>834 ± 424 (5 mg ambrisentan)</td>
</tr>
<tr>
<td></td>
<td>912 ± 465 (10 mg ambrisentan)</td>
</tr>
</tbody>
</table>

PAP: pulmonary arterial hypertension; 6MWD: 6-minute walk distance; PVR: pulmonary vascular resistance; CI: cardiac index; mPAP: mean pulmonary artery pressure.
Endothelin receptor antagonists in pulmonary medicine  

Sitaxentan  

Sitaxentan has not been approved by the FDA for the treatment of PAH in the United States. It was authorized in the European Union in 2006 for the treatment of NYHA III functional class PAH, and in Canada and Australia in 2007 for functional class II and III PAH. It has been marketed in France since 2007 (Thelin®, Encysive, currently Pfizer) at a single oral dose of 100 mg per day.

Pharmacological data  

Sitaxentan is currently the most selective ETA antagonist with an affinity 6500 times greater than that for ETB [64].

Pharmacokinetics  

Sitaxentan has an oral bioavailability independent of food intake and in particular a long half-life, between 5 and 7 hours, allowing once-daily oral administration [65]. It has an inductive action vis-à-vis CP450, in particular, it inhibits CYP2C9 activity, which increases blood concentrations of other drugs metabolized by this isozyme, especially warfarin [65]. Dose adjustment is not required in renal failure [66]. The potential liver toxicity of the drug justifies regular surveillance of transaminase levels [67]. In the initial clinical study, sitaxentan was administered at doses of 100 to 500 mg [67]. Two cases of acute hepatitis, including one of fatal fulminant hepatitis, occurred during the extension phase. In both cases, the dose of sitaxentan (600 mg/day) was higher than that recommended.

Drug interactions  

They mainly concern interaction between warfarin and sitaxentan. In the initial STRIDE-2 study, an 80% reduction in warfarin doses had been necessary at the start of treatment [68]. An unpublished post-hoc analysis showed that warfarin doses were higher in the bosentan versus the sitaxentan group, with similar adjustments between the two groups [69].

Clinical data  

The efficacy and tolerance of sitaxentan in PAH have been the subject of a programme called “STRIDE” for “Sitaxentan To Relieve Impaired Exercise”, including three pivotal randomized placebo-controlled studies (STRIDE-1, STRIDE-2 and STRIDE-4) (Table 3), two uncontrolled studies (STRIDE-6 and Study 211), and three long-term studies (STRIDE-1X, STRIDE 2X and STRIDE-3). The first study (STRIDE-1) was conducted in the United States and Canada, in 178 patients with idiopathic PAH (53%) or PAH associated with connective tissue disease or congenital heart disease, randomized into three groups: sitaxentan 100 mg or 300 mg/day and placebo [70]. The primary endpoint, peak VO₂ during exercise testing, only showed a significant improvement of 3% in the group treated with 300 mg. However, the 6MWD and haemodynamic parameters improved similarly in both treatment groups versus placebo. Quality of life, time to clinical worsening, and NYHA functional class were unchanged. This can be partly explained by the fact that 33% of the patients were NYHA class II, and 6MWD was significantly greater than in studies with bosentan (398 ± 110 m) [25,26]. The incidence of increased transaminase levels was 3% and 10% respectively in the 100 and 300 mg groups. There was an increase in anticoagulant effect in both treatment groups versus placebo, which resolved after reducing the warfarin doses. The choice of primary endpoint for this study may seem surprising, especially as inter-hospital variability has been demonstrated retrospectively for this parameter [71]. The STRIDE-2 study was conducted similarly in 247 randomized patients (50 and 100 mg/day of sitaxentan or placebo) [68]. The functional and haemodynamic parameters showed less changes than previously. At 18 weeks of treatment, 6MWD and NYHA functional class had improved in the 100 mg group, but not in the 50 mg group. There was also a subgroup of 60 patients, treated with open-label bosentan, for which the improvement in 6MWD was similar to that in the sitaxentan group. However the open nature of the study does not allow for comparison of results.

The unpublished STRIDE-X open study to evaluate long-term treatment with sitaxentan was based on the STRIDE-2 study with 40 ± 15 weeks follow-up [72]. Patients treated with 50 mg/day of sitaxentan in STRIDE-2 saw their dose increased to 100 mg/day, those already taking bosentan continued this treatment, and those receiving the placebo,
## Table 3 Published pivotal studies on sitaxentan in PAH.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>No. patients</th>
<th>Treatment</th>
<th>Initial NYHA stage</th>
<th>Primary endpoint results</th>
<th>Results Haemodynamic</th>
<th>Other criteria</th>
<th>Tolerance Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barst 2004 [70] STRIDE-1</td>
<td>R, DB, PC 12 W</td>
<td>178 adults PAH, c, chd</td>
<td>100 mg or 300 mg × 1/day sitaxentan versus placebo</td>
<td>II (33%) II (37%) III (66%) IV (1%) IV (4%)</td>
<td>+ 3% VO₂ max compared to the theoretical norm for the 300 mg group (ns for the 100 mg group)</td>
<td>Improvement in all parameters (ns for the 100 mg group)</td>
<td>6MWD +35 m (100 mg) +33 m (300 mg) Improvement in NYHA 100 mg group (29%) 300 mg group (30%)</td>
<td>↑ Transaminases in 300 mg group (10%) ↑ INR (19%) Oedema (21%)</td>
</tr>
<tr>
<td>Barst 2006 [68] STRIDE-2</td>
<td>R, DB, PC + Bosentan arm in OS 18 W</td>
<td>247 adults PAH, c, chd</td>
<td>50 mg or 100 mg × 1/day sitaxentan or 1 Bosentan arm according to SPC versus placebo</td>
<td>II (37%) II (37%) III (59%) IV (4%)</td>
<td>6MWD + 17.8 m (50 mg) + 24.9 m (100 mg) + 23 m (Bosentan OS)</td>
<td>NP</td>
<td>Improvement in NYHA 100 mg group (13%) (ns for the other groups)</td>
<td>↑ Transaminases 50 mg group (5%) 100 mg group (3%) Bosentan OS (11%)</td>
</tr>
</tbody>
</table>

- **R**: randomised; **DB**: double-blind; **PC**: placebo-controlled; **OS**: open study; **SD**: standard deviation; **W**: weeks; **6MWD**: 6-minute walk distance; **NP**: not performed; **(ns)**: not statistically significant; **PVR**: pulmonary vascular resistance; **CI**: cardiac index; **RAP**: right atrial pressure; **mPAP**: mean pulmonary artery pressure; **PAH, c, chd**: PAH, idiopathic or with connective tissue disease, or with congenital heart disease; **INR**: international normalized ratio; **SPC**: summary of product characteristics.
100 mg of sitaxentan or bosentan. At one year of treatment, the improvement in 6MWD was maintained, but without significant intergroup differences. There was a trend towards less clinical worsening and increased survival in the sitaxentan group; this was more pronounced in the connective tissue disease subgroup. Post hoc analysis of the same subgroup in the STRIDE-1 study confirmed the improvement in 6MWD compared with the placebo group [73]. Nevertheless, given the small cohorts in both groups and the heterogeneous nature of the treatment group (100 or 300 mg), the long-term results require confirmation for this indication. Finally, STRIDE-6 was a randomised, double-blind, but not placebo-controlled study to determine the effectiveness of sitaxentan (50 and 100 mg) in 48 patients considered to be in treatment failure with bosentan [74]. Bosentan had been discontinued because of inadequate efficacy (n = 35) or liver or skin intolerance (n = 13). In the group treated with 100 mg, 5 of the 15 patients who had discontinued bosentan because of inefficacy had improved their 6MWD by +15% versus 2 of the 20 in the group treated with 50 mg. Only one patient who had discontinued bosentan because of hepatic cytolysis presented a similar complication with sitaxentan. Though the subject of the study was interesting, the absence of a placebo group was regrettable, as was the lack of precision concerning treatment with bosentan, both its duration and its inefficacy as established by the investigator.

Finally, given the limited number of publications, studies that often included small cohorts, and the heterogeneous treatment groups, the results of sitaxentan in PAH require confirmation, particularly in the long-term. The results of the pivotal studies were considered inadequate for FDA approval; another randomized placebo-controlled study is currently in progress (STRIDE-5).

- Sitaxentan is currently the most selective ETA antagonist.
- It is administered once daily and inhibits oral anticoagulants.
- Sitaxentan is especially effective on 6MWD.

ERAs thus play an essential role in the treatment armamentarium of PAH. The survival benefit in the long term needs to be clarified, particularly for ambrisentan and sitaxentan. The clinical impact of ETA and ETB receptor selectivity is yet to be demonstrated. The role of combination therapies also requires definition. Finally, the use of ERAs in early disease (NYHA functional class I or II), which is a promising strategy in the prognosis of PAH, must be confirmed subsequently with prolonged studies.

**ET-1 receptor antagonists (ERAs) in pulmonary diseases without vascular involvement**

In addition to its vasoconstrictor activity, ET-1 has pro-inflammatory, pro-fibrotic and mitogenic potentialities for smooth muscle cells, myocytes and fibroblasts. Moreover, it may be involved in the process of angiogenesis and contribute to endothelial cell apoptosis. Endothelin could thus play a pivotal role, to varying degrees, in pathological conditions related to fibroblast proliferation and collagen synthesis [75,76]. Increased production of ET-1 has moreover been described in cardiovascular disease (heart failure, atherosclerosis) and in some connective tissue diseases [77–81]. Several years ago, an increase in circulating endothelin concentration [78], and in expression of prepro ET-1 mRNA in skin fibroblasts [79], had already been observed in patients with systemic sclerosis compared with healthy control subjects, with a positive correlation between ET-1 level and fibrotic phenotype severity. Furthermore, in a population of 26 patients with systemic sclerosis an increase of approximately 25% (range 5–47%) was found in median expression levels of ET-1 in the bronchoalveolar lavage fluid of these patients compared to controls (p < 0.02), with a significant increase in expression for patients with pulmonary fibrosis (n = 16) compared with those not presenting pulmonary fibrosis (n = 10) (p < 0.05) [80]. These results suggest that ET-1, produced in excess by abnormally functioning fibroblasts in systemic sclerosis, plays a role in cutaneous sclerosis, Raynaud syndrome, and the pulmonary fibrosis observed in the disease [81].

In this context, considering the role played by ET in the cascade of events leading to fibrosis, it was legitimate to question the potential role of ERAs in the treatment of idiopathic pulmonary fibrosis (IPF), with its known particularly poor prognosis [82], in the absence of a genuinely effective treatment to date [83,84]. The current reference treatment for IPF is mainly based on corticosteroids, alone or associated with azathioprine and N-acetylcysteine, and, in the case of treatment failure, with immunomodulatory agents such as cyclophosphamide, with disappointing results [85,86]. More recently, other drugs with antiproliferative potential have emerged such as interferon gamma and pirfenidone, here again with insufficient and contradictory data, or others not yet evaluated such as protein tyrosine kinase inhibitors [87–90]. These developments in treatment obviously take into account progress made in the understanding of the pathophysiology of IPF, in particular the vital role played by complex interactions between epithelial cells and lung fibroblasts [91–93]. Alveolar epithelial cells express an increased amount of ET-1 that participates in fibroblast proliferation, epithelial-mesenchymal transition, and thus the production of extracellular matrix in IPF [93–96].

Considering the demonstration that bosentan reduces collagen production in alveoli in bleomycin-induced fibrosis in the rat [97], the need for clinical evaluation of this drug in humans became necessary for this indication. The first Bosentan Use in Interstitial Lung Disease (BUILD-1) multi-center, randomised, placebo-controlled trial was published in 2008 [98]. One hundred and fifty eight patients with IPF according to the criteria of the American Thoracic Society and the European Respiratory Society, and not presenting PAH, were included [98]. There was no difference in 6MWD between the treatment and the placebo groups. However, there was a trend towards a lower risk of functional deterioration in the treatment (22.5%) versus the placebo (36.1%) group with a relative risk (RR) of 0.62 (CI 95%: 0.37–1.05). When focusing on the subgroup of 99 patients with documented histology by surgical biopsy, the difference became
significant with a reduced risk of death or worsening of disease in the bosentan group (RR 68%, CI 95%: 21—86%). It should however be emphasized that a lung biopsy had been proposed when the CT scan appearance was less typical (presence of ground glass opacities or scarcity of honeycomb lesions), with less extensive or less developed lesions, thus in patients less severely affected. In the a posteriori analysis of the BUILD-1 study, unpublished to date, the incidence of fibrosis progression or the occurrence of death was 3.8% in the bosentan group versus 42.8% in the placebo group in patients with atypical thoracic CT scan (reduction of RR to 91%), and 11.4% versus 34.8% respectively in the treatment and placebo groups in those with a “typical” CT scan (reduction of RR to 68%) [99]. These data suggested a greater efficacy of bosentan at an early stage of the fibrosis process, when the lesions are not yet definitively fixed, provided the basis for the BUILD-3 trial currently underway [100].

In this multicenter randomised double-blind study of bosentan versus placebo, the primary endpoint is the time to death or worsening of the disease, and can only include patients with histologically proven IPF with little or no fixed CT scan abnormalities (such as “honeycomb” images). The BUILD-2 study, conducted in pulmonary fibrosis associated with systemic sclerosis showed results comparable to those obtained in BUILD-1 [101]. Thus current data do not provide a conclusion on the role of bosentan in the treatment of IPF or in association with systemic sclerosis, but its use in pulmonary fibrosis, particularly in its early stage, probably merits evaluation.

Other ERAs are currently in Phase II and III trials, particularly in disorders with a vascular component (renal vascular disease, acute decompensated heart failure, terminal renal failure, or cerebral aneurysm) but not in IPF [102]. Most are selective ETA receptors: atrasentan, alogsetan, clazosentan, darusentan, and edonentan, with some mixed ERAs: tizosentan, and recently macitentan [102].

**KEY POINTS**

- ET-1 has pro-inflammatory, pro-fibrotic and miogenic potentialities for smooth muscle cells, myocytes and fibroblasts.
- In idiopathic pulmonary fibrosis, ERAs did not modify 6MWD, but the risk of functional worsening was less in the treatment group.
- Bosentan seems to be more effective in the early stage of fibrosis, when the lesions are not yet definitively fixed.

**Conclusion**

Its potent vasoconstrictor properties and its role in pulmonary vascular remodeling have made ET-1 a major mediator in the pathophysiology of pulmonary arterial hypertension. The clinical benefit, tolerance and ease of oral administration of ERAs have been widely demonstrated, making them an integral part of the current treatment armamentarium for PAH. The recent acknowledgment of the role of ET in fibrogenesis and angiogenesis, and its pleiotropic nature, also make ERAs potentially useful drugs in various disorders where fibrosis is preponderant, primarily in idiopathic pulmonary fibrosis and fibrosis associated with connective tissue disease. Although many questions remain, this new class of ERAs suggests the possibility of a broad therapeutic field, whose indications will be specified by future trials.

**References**


[56] Effects of the combination of Bosentan and Sildenafil versus Sildenafil monotherapy on pulmonary arterial hypertension (Compas2), NCT00303459, Clinicaltrials.gov.


[60] Duffon C, Gerber MJ, Yin O. Non clinically relevant pharmacokinetic interaction between ambrisentan and sildenafil. Chest 2006;130:254S.

[61] Gerber MJ, Duffon C, Pentikis H. Ambrisentan has no clinically relevant effect on the pharmacokinetics or pharmacodynamics of warfarin. Chest 2006;130:256S.


[69] Coyne T, Dixon R. Warfarin management in pulmonary arterial hypertension is similar between bosentan, placebo, and sitaxentan. Chest 2005;128:366S.


