ACE2 as therapy for pulmonary arterial hypertension: the good outweighs the bad

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Based on this highly encouraging clinical proof-of-concept, there is now a clear plan to explore interventions that stimulate the ACE2–Ang-(1–7)–Mas pathway further in large clinical trials
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Introduction

Since its initial discovery 120 years ago, the understanding of the renin–angiotensin system (RAS) has advanced considerably. In recent years, the identification of new enzymes, peptides and receptors that are constituents of novel identified counterbalancing RAS pathways acting beyond the classical ACE (angiotensin-converting enzyme)/angiotensin (Ang)II/AT1 receptor axis have heralded a new era highlighting its importance in health and disease (figure 1). As a result, it is now well established that inhibition of the classical RAS and activation of the counterbalancing axes (alternative RAS), namely the ACE2–Ang-(1–7)–Mas receptor cascade and the AT2 signalling pathway are two feasible strategies demonstrating efficacies in various cardiovascular and pulmonary disease models, including in various preclinical models of pulmonary hypertension (PH) [1–6].

In this issue of *European Respiratory Journal*, Hemnes *et al.* [7] present the results of a study that provides evidence that shifting the RAS towards its alternative RAS pathway through infusion of a single dose of recombinant human ACE2 (GSK2586881; 0.2 mg·kg^{-1} or 0.4 mg·kg^{-1} *i.v.*, NCT01884051) is well-tolerated and may have potential haemodynamic benefits in pulmonary arterial hypertension (PAH).

Classic and alternative RAS pathways

When renal blood flow is reduced, juxtaglomerular cells in the kidneys secrete renin, an aspartyl protease, in the circulation that cleaves circulating angiotensinogen produced by the liver to form the inactive decapeptide AngI (Ang-(1–10)) from the amino-terminal portion of the protein. Then, the C-terminal dipeptide of AngI is predominantly cleaved by ACE (also known as ACE1, a large protein of 200 kDa) that is mainly located at the endothelial surface of pulmonary vessels, giving rise to the bioactive peptide AngII (Ang-(1–8)). However, AngI is also known to be a substrate of several other enzymes, such as cathepsins D and G, tonin and tissue plasminogen activator that may also form this main active effector of the classical RAS, AngII (and its active derivative peptides) [8, 9]. When AngII is produced, it binds to the G protein coupled receptor AT1 that is known to trigger a broad range of biological effects including aldosterone secretion, salt and water retention, inflammation and potent arteriolar vasoconstriction (figure 1).

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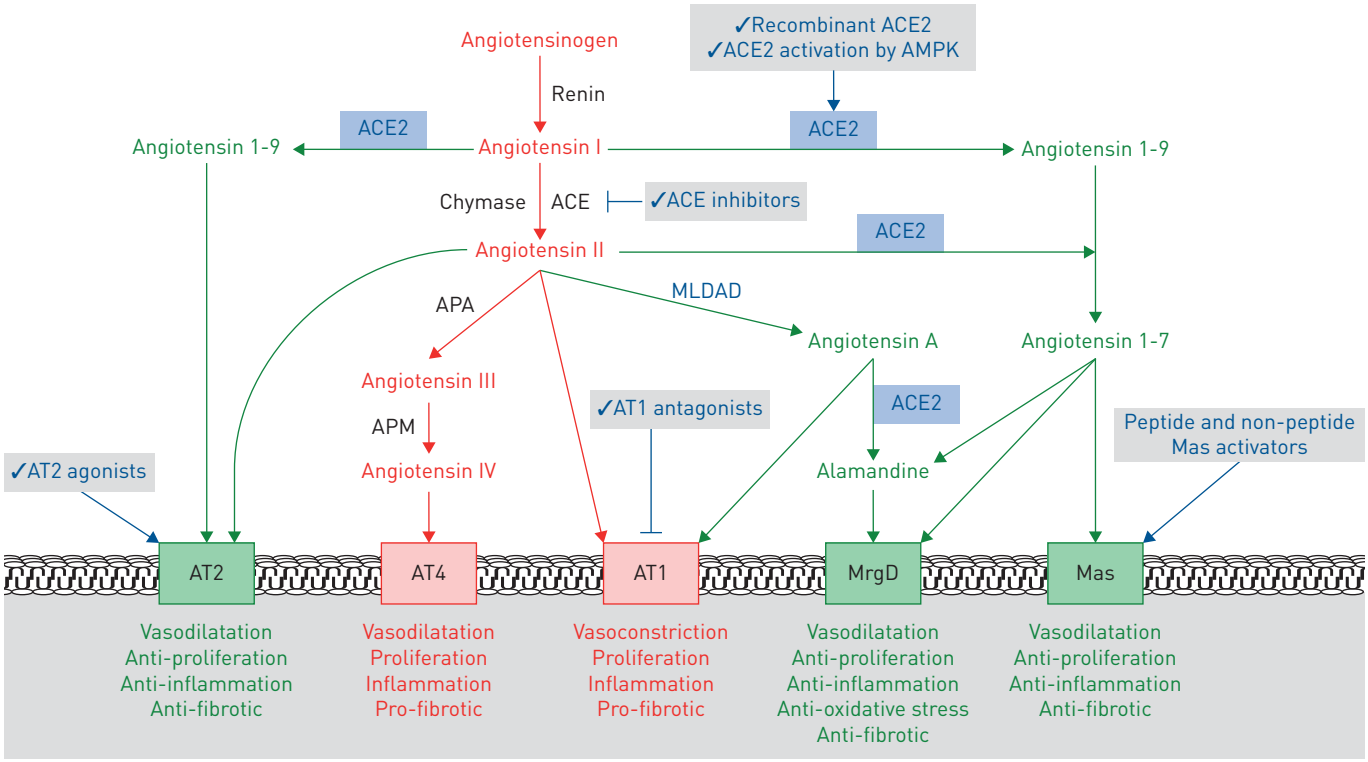

FIGURE 1 Schematic illustration of the renin–angiotensin system (RAS). The ACE2–Ang-(1–7)–Mas axis (green lines) counterbalances the harmful effects of the ACE1–Ang II–AT1 axis (red lines). ACE: angiotensin-converting enzyme; APA: aminopeptidase A; APM: aminopeptidase M; AT1: angiotensin type-1 receptor; AT2: angiotensin type-2 receptor; AT4: angiotensin type-4 receptor; Mas: Mas receptor; MrgD: Mas-related G protein coupled receptor; MLDAD: mononuclear leukocyte-derived aspartate decarboxylase.

While the majority of AngII actions are mediated *via* AT1, the ability of another G protein coupled receptor AT2 to bind AngII leads to vasodilation instead of vasoconstriction and thus represents the first known counterbalancing RAS pathway. Furthermore, the discovery that these proteolytic cleavages can also occur outside from the blood circulation in a number of intrinsic tissue-specific RAS systems has led to the concept of tissue RAS that can independently and locally control the circulating system in mediating diverse physiological functions.

Since its identification in 2000 [10, 11], the carboxypeptidase ACE2 has also received growing interest in several disease contexts and is currently representing the second counterbalancing RAS pathway. ACE2, which shares approximately 61% homology sequence with the catalytic domains of its homologue ACE1, primarily hydrolyses AngII into two biologically active products of the RAS cascade, namely Ang-(1–9) and Ang-(1–7) with high efficiency. Indeed, both Ang-(1–9) and Ang-(1–7) promote vasodilation and decrease cell growth and inflammatory responses (figure 1). As compared to ACE1, ACE2 exhibits a more restricted distribution pattern, mainly in the heart, kidney and lungs. Interestingly, ADAM17 (a disintegrin and metalloproteinase 17; also known as tumour necrosis factor α-converting enzyme) can cause shedding of ACE2 from the cell membrane [12]. Based on this knowledge, several anti-hypertensive, cardiovascular and renoprotective drugs are currently under development and clearly pave the way of novel innovative therapeutic strategies for several diseases, including in PAH, as underlined in this issue of *European Respiratory Journal* by Hemnes *et al.* [7].

Dysregulation of circulating and local RAS and potential therapeutic applications in PAH

Several recent studies have highlighted dysregulation of circulating and local tissue RAS in the serum and within remodelled arterial walls in lungs of patients with various form of PAH. De Man *et al.* [2] found increased serum levels of renin, AngI, and AngII and correlations with disease progression and mortality in a population of 58 patients with idiopathic PAH (iPAH). These authors also documented that pulmonary endothelial cells derived from iPAH patients produced significantly more AngII after AngI exposure than control endothelial cells, a phenomenon that was found to be totally abolished by coincubation with the ACE inhibitor enalapril. Consistent with these observations, Orte *et al.* [13] have

reported the greatest intensity of ACE immunostaining in intra-acinar small pulmonary arteries in explanted lung sections from iPAH patients when compared to control subjects. Since an upregulation of AT1 receptor expression and signalling has been reported in pulmonary arterial smooth muscle cells (PASMCs) of remodelled vessels in lungs of patients with iPAH without changes in AT2 receptor expression [2], this inappropriate local overproduction of AngII by the dysfunctional pulmonary endothelium was likely to be important for the progression of the pulmonary vascular remodelling. This notion was further supported by results from multiple animal studies that have indeed underscored beneficial effects of several AT1 receptor blockers (*e.g.* losartan, telmisartan, olmesartan) [2, 4, 14, 15], ACE inhibitors (*e.g.* captopril, enalapril) [5, 16, 17], and of AT2 agonists (*e.g.* compound 21) [18]. These promising preclinical data have encouraged a double blind placebo controlled trial of losartan in patients with PH associated with chronic obstructive pulmonary disease [19]. This trial was unfortunately statistically underpowered and although a *post hoc* analysis suggested benefit in patients with severely elevated pulmonary hemodynamics, no firm clinical improvements were noted.

In this issue of the *European Respiratory Journal*, Hemnes *et al.* [7] found a relative imbalance between the classical and alternative RAS pathways in PAH. Increased AngII levels without changes in Ang-(1–7) levels were found in the serum of 11 idiopathic or heritable PAH patients [7]. Decreased levels of serum ACE2 and Ang-(1–7) in patients with PAH due to congenital heart disease were also reported [20, 21]. Consistent with this notion of an imbalanced ACE/ACE2 ratio in PAH patients, Zhang *et al.* [3] have recently reported that the expression and phosphorylation status (Ser-680) of ACE2 were markedly decreased in remodelled pulmonary arteries in explanted lung sections from patients with iPAH. Consistently, these authors demonstrated that mice deficient in ACE2 ($ACE2^{-/-}$ mice) are more prone to develop chronic hypoxia-induced PH than wildtype littermates, whereas mice with a gain-function in ACE2 activity (ACE2 S680D knock-in mice) are protected [3]. Consistent with these findings, beneficial effects of ACE2 gene transfer (with lentiviral vector [22] or lentiviral packaged Ang-(1–7) fusion gene or ACE2 cDNA [23]) and of several ACE2 agonists (*e.g.* Ang-(1–7), cyclic analogue of Ang-(1–7), diminazene, XNT, resorcinolnaphthalein, AVE0991, NCP-2454) [1, 24–28] against experimental PH have been reported in various preclinical models. In addition, oral delivery of ACE2 and Ang-(1–7) bioencapsulated in plant cells may also prevent or reverse monocrotaline-induced PH [29]. Hemnes *et al.* [7] have pushed these preclinical results even further by showing that infusion of a single dose of recombinant human ACE2 (GSK2586881; 0.2 $mg \cdot kg^{-1}$ or 0.4 $mg \cdot kg^{-1}$ *i.v.*, NCT01884051) is well-tolerated and efficient in improving cardiac output and pulmonary vascular resistance in PAH patients.

Challenges and opportunities in clinical translation of ACE2 infusion as therapy for PAH

These promising outcomes raise a number of questions concerning underlying mechanism and clinical applicability.

Clinically, it remains unknown which patients may benefit most from ACE2 infusion and whether these beneficial effects obtained by infusion of recombinant human ACE2 in PAH might apply to other subgroups of patients with PAH or PH. Estradiol, *via* ERα, is a known modulator of the ACE/ACE2 and AT1/AT2 receptor [30, 31]. In addition, the role of ACE2 on right ventricular adaptation is unclear, although recombinant human ACE2 has also been shown to improve right ventricular function in a pressure overload model [32]. Finally, a long-term follow-up studies of immunogenicity and safety of this biotherapeutic approach and their potential impacts on arterial blood pressure, renal function and the central nervous system are required. In addition, advantages of direct local administration of ACE2 into the lungs or the use of a targeted drug delivery could be also considered. The recent demonstration that AMP-activated protein kinase phosphorylation of ACE2 Ser-680 increases ACE2 stability could also represent an alternative to the use of recombinant human ACE2 [3]. Since AngII could induce interleukin (IL)-6 through a mineralocorticoid receptor (MR)-dependent mechanism [33], MR antagonists and agents targeting IL-6 or its receptor IL6R, that is ectopically expressed on PASMCs in PAH [34], could also represent an alternative therapeutic strategy.

In addition, these findings raise questions concerning the exact mechanisms by which infusion of recombinant human ACE2 exerts its beneficial effects. In accordance with the anti-inflammatory and antioxidative role of the AT2 receptor [35], the study of Hemnes *et al.* [7] revealed rapid reduction of CCL2 (monocyte chemoattractant protein 1) and TGF-β/Smad repressor TG-interacting factor 1 within 2–4 h and increased superoxide dismutase 2 plasma protein at 2 weeks. Nevertheless, the mechanism through which ACE2 plays a role in inflammatory lung disease has not been clearly identified. Indeed, ACE2 is a multifunctional enzyme with several non-enzymatic actions [36]. In line with this notion, administration of ACE2 blunts the rise in pulmonary arterial pressure that occurs in response to hypoxia partly through stimulation of endothelium-dependent NO release [37–39]. In addition, ACE2 is known to

hydrolyse dynorphin A (1–13), apelin-13 and des-Arg(9) bradykinin. However, the role of ACE2 in these peptide systems is still obscure and needs to be solved. Further investigation is required to better understand the relationship between ACE2 and placental growth factor, a pro-angiogenic molecule that is selectively involved in pathological angiogenesis and that promotes expression of the pulmonary vasoconstrictor endothelin-1 [40]. Another emerging challenge is related to the stability, half-life and bioavailability of ACE2. Indeed, ACE2 contains a catalytically active ectodomain that may be cleaved by ADAM-17 and other proteinases [12].

Concluding remarks

Even if there is clearly much more work to be done in this area, there is compelling evidence that stimulation of the ACE2−Ang-(1–7)−Mas pathway represents a potential pharmacological target for PAH. Along this line, the translational study by Hemnes *et al.* [7] demonstrates that intravenous administration of recombinant ACE2 (GSK2586881) is tolerated, and a beneficial haemodynamic effect in patients with stable PAH on background therapies is suggested. Based on this highly encouraging clinical proof-of-concept, there is now a clear plan to explore this, or other related alternative interventions, further in large clinical trials. The main challenge is to better understand the exact mechanisms by which infusion of recombinant human ACE2 produces its beneficial effects. Undoubtedly, these findings will have significant impact for future investigations in the field and in other cardiovascular disorders.

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