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Review Article

Endothelin System and Therapeutic Application of Endothelin Receptor Antagonists

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ABSTRACT

Endothelin is a 21 amino acid molecule endogenous potent vasoconstrictor peptide. Endothelin is synthesized in vascular endothelial and smooth muscle cells, as well as in neural, renal, pulmonic, and inflammatory cells. It acts through a seven transmembrane endothelin receptor A (ETA) and endothelin receptor B (ETB) receptors belongs to G protein-coupled rhodopsin-type receptor superfamily. This peptide involved in pathogenesis of cardiovascular disorder like (heart failure, arterial hypertension, myocardial infraction and atherosclerosis), renal failure, pulmonary arterial hypertension and it also involved in pathogenesis of cancer. Potentially endothelin receptor antagonist helps the treatment of the above disorder. Currently, there are a lot of trails both per-clinical and clinical on endothelin antagonist for various cardiovascular, pulmonary and cancer disorder. Some are approved by FAD for the treatment. These agents are including both selective and non-selective endothelin receptor antagonist (ETA/B). Currently, Bosentan, Ambrisentan, and Macitentan approved for the treatment of pulmonary arterial hypertension. The aim of this review is to introduce endothelin system and its antagonist drugs with their detail pharmacological and pharmacokinetics profile.

Key words: Endothelin system; Antagonist; Therapeutic indication

Abbreviations/Acronym: EDCF: Endothelin-derived contracting factor; ET: Endothelin; ECE: Endothelin Converting Enzyme; LDL: Low Density lipoprotein; ETA: Endothelin Receptor Type A; ETB: Endothelin Receptor Type B; NO: Nitric Oxide; PGE: Prostaglandin E2; PAH: Pulmonary Artery Hypertension; EMA: European Medicine Agency; INR: International Normalization Ration

INTRODUCTION

Background

Subsequently, research in the early 1980s was concentrated on identifying new vasoactive molecules [1,2]. Work of several laboratories from the mid-1980s identified a potent vasoconstrictor substance released into the supernatant of endothelial cells [3]. In 1988, Masashi Yanagisawa and his colleagues discovered potent vasoconstrictor peptide which produced by vascular endothelial cells and they were published their work in Nature [4]. After ten years in 1998, Tomoh Masaki identified the peptide named endothelin-1 in Japanese [5].

The events that led to the discovery of the peptidergic endothelium-derived contracting factor (EDCF, later identified as endothelin) started in the fall of 1982, 6 years after the discovery of the first endothelial vasoactive substance, prostacyclin [6] and 2 years after the report by Furchgott and Zawadzki of the obligatory role of the endothelium in vaso-relaxation induced by acetylcholine [2]. That period in the late 70s and early 80s represented one of the most exciting and productive years in the history of vascular biology, when a completely novel mechanism of regulation of vascular function and structure started to emerge: the active role of endothelium and endothelium-derived vasoactive substances [7].

After the discovery of the endothelium-derived relaxing factor a contracting factor was isolated from bovine aortic and pulmonary endothelium [8]. Its gene sequence was identified in 1987, and it was named endothelin (ET) [4]. ET is a family of four 21-amino acid peptides, ie, ET-1, ET-2, ET-3 and ET-4 (vasoactive intestinal constrictor) [9]. In addition, 31-residue ETs have also been identified. ET-1, the predominant isoform, has a striking similarity to the venom of snakes of the Atractaspis family and it is a potent vasoconstrictor [10]. In addition to their cardiovascular effects, ETs are involved in embryonic development, bronchoconstriction,

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prostate growth, carcinogenesis and gastrointestinal and endocrine function [11].

The Endothelin System

Endothelin and its biosynthesis: Endothelin is synthesized in vascular endothelial and smooth muscle cells, as well as in neural, renal, pulmonic, and inflammatory cells. It consists of four closely related peptides, endothelin (ET)-1, ET-2, ET-3, and ET-4. These peptides are converted by endothelin-converting enzymes (ECE-1 and -2) from 'big endothelins' originating from large pre-pro-endothelin peptides cleaved by endopeptidases [12]. ET-1 is the principal isoform in the human cardiovascular system and remains the most potent and unusually long lasting constrictor of human vessels so far discovered. In the endothelium, ET-1 is released abluminally toward the vascular smooth muscle, suggesting a paracrine role [3, 4]. It also produced by other cells involved in vascular disease, such as leukocytes, macrophages, smooth muscle cells, cardiomyocytes, mesangial cells and its synthesis is regulated in an autocrine fashion [13]. In endothelial cells, shear and mechanical stress increase ET release [14]. In addition, a variety of other factors, including vasopressin, angiotensin II, catecholamine, pro-inflammatory cytokines (e.g., tumor necrosis factor-α and interleukin-1 and -2), hypoxia, and oxidized low-density lipoprotein particles have been shown to stimulate production of ET [15]. Release of ET systemically and within the heart is increased in a variety of pathologic settings that lead to or are associated with cardiac dysfunction [16]. Moreover, the cellular effects of ET, including constriction of vascular smooth muscle, cell proliferation, hypertrophy of cardiac myocytes, and activation of cardiac fibroblasts, are those associated with both the clinical manifestations of heart failure, pathologic remodeling of the heart that results in progressive cardiac dysfunction and excessive cell proliferation [17]. In synthesis of endothelin first the precursor pre-pro-endothelin converted to big endothelin molecule by endopeptidase enzyme then converted to a 21 amino acid molecule (endothelin) by using endothelin converting enzyme [4]. Bioactive endothelins are the product of posttranslational processing of the parent pre-pro-endothelin peptide. Transcription of the pre-pro-endothelin gene and translation of pre-pro-endothelin mRNA results in the formation of this 203-amino acid peptide, which is later cleaved by a furin convertase to the 38-amino acid peptide big ET 1–38 [18]. Then, big ET is further processed into ET 1–21 by different isoforms of endothelin converting enzymes (ECEs), a group of proteases that belong to the metalloprotease family and share both structural and functional similarity with neutral endopeptidases and Kell blood group proteins. In alternative way, bioactive 31-amino acid length ETs, ETs(1-31), are also synthesized by chymases (proteases present in large quantities in mast cell and smooth muscle cells), and non-ECE metalloproteases that selectively cleave big ET-1, -2 and -3 at their Tyr31-Gly32 bonds [19].

Regulation: Transcription of the pre-pro-endothelin gene is regulated through the phorbol-ester–sensitivec-fos and c-jun complexes, acute phase reactant regulatory elements, and binding sites for nuclear factor-1, AP-1, and GATA-2 [20]. Endothelin synthesis is regulated by physicochemical factors such as pulsatile stretch, shear stress and PH. Exercise upregulates myocardial ET-1 expression, which suggests ET-1 may play a role in maintaining cardiac function [21]. Hypoxia is a strong stimulus for ET-1 synthesis that may be important in ischemia. ET-1 biosynthesis is stimulated by cardiovascular risk factors such as elevated levels of oxidized LDL cholesterol and glucose, estrogen deficiency, obesity, cocaine use, aging, and pro-coagulant mediators such as thrombin [20,22]. Furthermore, vasoconstrictors, growth factors, cytokines, and adhesion molecules also stimulate ET production. Inhibitors of ET-1 synthesis include nitric oxide (NO), prostacyclin, atrial natriuretic peptides and estrogens [23].

Endothelin receptor: Majorly endothelin receptor widely expressed in tissues of the cardio- and reno-vascular system. In addition to these it also expressed in almost all of the remaining physiological systems, such as central and peripheral nervous system, respiratory and digestive tracts, genitourinary system and endocrine glands [24]. According to International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR), endothelin receptor classified under two types and named as ETA and ETB endothelin receptor [25]. In human vasculature, ETA receptors predominate on the smooth muscle cells, and a low density of ETB receptors (<15%) is also present on these cells. In vitro studies show a discrete participation of ETB receptors on the vaso-constricting response [26]. However, in vivo studies investigating the effects of exogenously infused ET-1 (as a non-selective agonist for ETA and ETB receptors) or Sarafotoxin S6c (as an ETB receptor agonist), suggest that both receptor types can mediate vasoconstriction in human resistance and capacitance vessels [27].

The structures of the mature ET receptors show that both ETA and ETB receptors belong to the seven-transmembrane domain or G protein-coupled rhodopsin-type receptor superfamily. The activation of ET receptors in smooth muscle cells results in phospholipase C activation which leads to the generation of the second messengers inositol trisphosphate and diacylglycerol, which can in turn stimulate intracellular calcium release and protein kinase C activation, respectively [26]. Other signaling pathways are also activated and involve phospholipase D and diacylglycerol generation, phospholipase A2 stimulation and arachidonic acid release, activation of the Na+-H+ exchanger, and activation of the mitogen-activated protein kinase (MAPK) cascade. Activation of all these signaling pathways are involved in the short term regulation of smooth muscle tone as well as in the long term control of cell growth, adhesion, migration and intercellular matrix deposition in the vasculature and the heart [24]. The activation of endothelial cell ETB receptors stimulates the release of nitric oxide (NO) and prostacyclin, negatively modulating the constrictor effects of ET-1 on smooth muscle cells [28].

The following figure showed that ET-1 receptor activation causes vasoconstriction and cell proliferation through activation of specific ETA receptors on vascular smooth muscle cells [23]. In contrast, ETB receptors on endothelial cells cause vasodilation via release of nitric oxide (NO) and prostacyclin; the overall effect of ET-1 at the ETB receptor is vasodilation [29]. Additionally, ETB receptors in the lung are a major pathway for the clearance of ET-1 from plasma. ETB receptors also contribute to the autocrine regulation of ET-1 synthesis [12]. The ETA receptor has a higher selectivity for ET-1 than for ET-2 and ET-3, whereas the ETB receptor is non-isopeptide selective [30]. The binding of ET-1 to its receptor causes an increase in intracellular calcium levels (Figure 1) [31].

Potential Therapeutic Indication

Cardiovascular disorder: Given the importance of the ET-1 pathway in the regulation of cardiovascular function and structure, it is not surprising that specific receptor antagonists have been

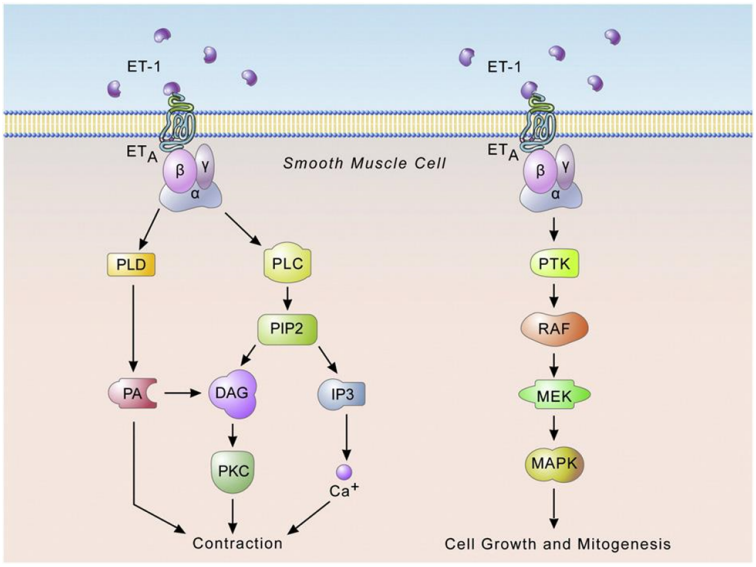

Figure 1: Type and activation cascade of endothelin receptor on isolated hepatic cell, Khimji A et al. 2011.

intensively studied in various clinical disorders. Indeed, endothelin receptor antagonism has been shown to be very promising for the pharmacotherapy of cardiovascular diseases such as arterial hypertension, atherosclerosis and congestive heart failure [12].

Arterial hypertension: Arterial hypertension is a strong risk factor for the development of atherosclerosis and heart failure. It is associated with dysfunction of the vascular endothelium i.e. an imbalance of endothelium-derived relaxing and contracting factors [13,32]. ET-1 acts as the natural counterpart to endothelium-derived NO, which exerts vasodilating, antithrombotic, and anti-proliferative effects, and inhibits leucocyte-adhesion to the vascular wall [33] Apart from its arterial blood pressure raising effect in humans, ET-1 directly induces vascular and myocardial hypertrophy [32].

Indeed, ET-1 plays an important role in human hypertension. Patients with hypertension exhibit an exaggerated sensitivity to endogenous ET-1 [16]. Furthermore, there is evidence that certain polymorphisms of the genes coding for ET-1 and endothelin receptors are associated with higher blood pressure levels [34]. Apart from the blood pressure-lowering effects of a specific antihypertensive drug, the actions of the drug on vascular function and structure are important. In animal models of hypertension, endothelial dysfunction is ameliorated and vascular and myocardial hypertrophy is prevented by treatment with an endothelin receptor antagonist [35].

Atherosclerosis: ET-1 essentially contributes to the pathogenesis of coronary artery disease, as ET-1 promotes direct vasoconstriction and induces smooth muscle cell proliferation through specific activation of ETA receptors [36]. Furthermore, ET-1 stimulates neutrophil adhesion and platelet aggregation [37] and functionally acts as a natural antagonist of endothelium-derived NO, a vasodilator with anti-proliferative and antithrombotic properties [38]. Oxidative modified low density lipoprotein–cholesterol induces the production of ET-1 by human macrophages and increases ET-1 release from endothelial cells [36]. Indeed, circulating and vascular tissue levels of ET-1 are elevated in patients with atherosclerotic vascular disease and correlate with the severity, i.e. the number of anatomic sites involved [38]. Staining for immunoreactivity ET-1 and ECE is enhanced in atherosclerotic plaques. Tissue ET-1 levels correlate with severity of angina pectoris in patients with coronary artery disease and increase as the clinical presentation becomes unstable [34]. Certain polymorphisms of the preproET-1 and receptor genes may indeed represent a predisposition for vascular diseases [38].

Experimentally, endothelin receptor blockade prevents endothelial dysfunction and structural vascular changes in atherosclerosis due to hypercholesterolemia [28]. Therefore ET-1 is an important determinant of coronary tone in coronary artery disease and may become an interesting therapeutic strategy both in the prevention and treatment of atherosclerotic vascular disease [12].

Myocardial infarction: Plasma ET-1 levels are greatly elevated in patients with acute myocardial infarction, and correlate with 1-year prognosis [39]. In experimental models of ischemia, treatment with an endothelin receptor antagonist reduced infarct size and prevented left ventricular remodeling after myocardial infarction [21]. When given to rats after myocardial infarction, treatment with the selective ETA receptor antagonist BQ 123 significantly improved survival [40].

Congestive heart failure: The strong vasoconstrictor action of ET-1 contributes to increased vascular resistance in heart failure through activation of ERA [38]. Both in experimental models as well as in patients with heart failure, circulating and cardiac tissue ET-1 levels are elevated [40]. However, the role of ETB receptors may differ in healthy volunteers and patients with heart failure. Whereas ETB receptors mediate vasodilation in healthy volunteers, ETB receptors may cause vasoconstriction, at least in the systemic circulation, in patients with heart failure [32].

Several mixed ETA/B receptor antagonists under clinical investigation for the treatment of congestive heart failure. A mixed ETA/B receptor antagonist like bosentan has been shown to improve systemic and pulmonary hemodynamics in patients with both acute and chronic congestive heart failure [41]. ET-1 contributes to the process of hypertrophy as it exerts growth-promoting effects on cardiomyocytes, thereby potentiating the effects of the renin angiotensin system [42]. To illustrate selective ETA blocker, has been shown to prevent the switch of myosin heavy chain isoform expression in an experimental model of heart failure. And also Aldosterone release depends on ETB receptor activation in some animal models of heart failure [39].

Renal failure: Systemic infusion of ET-1 in healthy volunteers leads to a decrease in renal plasma flow and glomerular filtration rate. Urinary sodium excretion is reduced by a decrease in both sodium and water reabsorption in the distal tubules [43]. Infusion of a selective ETA receptor antagonist 1 day after induction of severe acute renal failure enhanced the tubular reabsorption of sodium, increased glomerular filtration rate, and led to an improved survival in a rat model. In an experimental model of congestive heart failure, bosentan, produced an increase in cortical and medullary blood flow [44].

Pulmonary hypertension: Pulmonary hypertension is characterized by a progressive increase in pulmonary vascular resistance, ultimately leading to right ventricular failure and death. ET-1 expression is increased in pulmonary tissue of patients with pulmonary hypertension [45]. Vascular endothelial cells mainly produce and secrete endothelin (ET-1) in vessels that lead to a potent and long-lasting vasoconstrictive effect in pulmonary arterial smooth muscle cells. Along with its strong vasoconstrictive action, ET-1 can promote smooth muscle cell proliferation. Thus, ET-1 blockers have attracted attention as an antihypertensive drug, and the ET-1 signaling system has paved a new therapeutic avenue for the treatment of PAH [46].

In general, binding of ET-1 to ETA and ETB receptors on pulmonary arterial smooth muscle cell (SMCs) promotes vasoconstriction, whereas activation of ETB receptors on ECs causes vasodilation through an increase in PGI2 and NO levels [45] as well as through circulating ET-1 clearance elevation [21]. However, ET-1 affinity for ETA receptors is 100 times higher than that of ET-3, whereas all three isoforms have the same affinity for ETB receptors. The high concentration of ET-1 causes vasoconstriction rather than vasodilation. This leads developing pulmonary arterial hypertension [46].

In cancer treatment

Role of ET-1 in cell growth: ET-1 is a powerful vasoconstrictor with mitogenic or comitogenic properties, which stimulates proliferation in vitro of fibroblasts, renal mesangial cells, smooth muscle and several tumor cell lines, including colorectal cancer [47]. Studies involving colorectal, ovarian and prostate cancers suggest that the receptor responsible for the ET-1 mitogenic action is ETA, which is probably upregulated. Activation of the ETAR by ET-1 mediates a signaling cascade, which promotes tumor cell growth, synergizing with other growth factors to cause cell proliferation (Figure 2) [48]. The following figure showed that when endothelin interacts with ETA and activates a G protein that triggers a parallel activation of several signal transducing pathways. This interaction can in fact activate multiple signal transduction pathways including phospholipase C activity with a consequent increase in intracellular Ca2+ levels, PKC, phosphati-dylinositol 3-kinase and MAPK (Figure 2) [47]. ETBR-mediated coupling in choriocarcinoma cell lines leads to the activation of the Ras/Raf-MAPK pathway. ET-1 causes EGFR transactivation which is partly responsible for MAPK activation by a ligand-dependent mechanism involving a non-receptor tyrosine kinase, such as Src. Recent work has reported that a specific ETAR antagonist can reduce the EGFR transactivation [48].ET-1 promotes DNA synthesis and cell proliferation in various epithelial tumor cells, including prostate, cervical and ovarian cancer cells. Synergistic interactions with other growth factors, including EGF, basic fibroblast growth factor (bFGF), insulin, IGF, PDGF, TGFs and IL-6, intensify mitogenic activity [49].

Role of ET-1 in angiogenesis: Angiogenesis, the formation of new vessels from existing vasculature, is an important early event in tumor progression which begins in premalignant lesions [40]. In the following figure illustrated that the activation of ETAR by ET-1 promotes tumor growth and progression by stimulating the production of the key angiogenic factor VEGF in response to hypoxia. ET-1 regulates various stages of neovascularization, including endothelial cell proliferation, migration, invasion, protease production and tube formation, and also stimulates neovascularization in vivo [50]. ET-1 increases VEGF mRNA expression and VEGF protein levels in a dose- and time-dependent manner, and does so to a greater extent under hypoxia. ET-1 stimulates VEGF production through the hypoxia-inducible factor HIF-1a and this mechanism might be responsible for increasing tumor angiogenesis. Degradation of HIF-1a is in fact reduced in ET-1-treated ovarian carcinoma cells under both hypoxic and normoxic conditions, indicating that the induction of HIF-1a protein production by ET-1 is due to enhanced HIF-1a stability. After ETAR activation by ET-1, HIF-1a protein levels are increased, the HIF-1 transcription complex is formed and binds to the hypoxia-responsive element binding site [48]. Under normoxic condition, ET-1 significantly stimulates the expression of COX-1 and -2 at mRNA and protein levels, COX-2 promoter activity and prostaglandin PGE2 production. PGE2 promotes angiogenesis and this effect is mediated by VEGF (Figure 3) [40].

Role of ET-1 in apoptosis: Recently, investigations into the role of the ET axis in apoptosis inhibition have provided evidence of

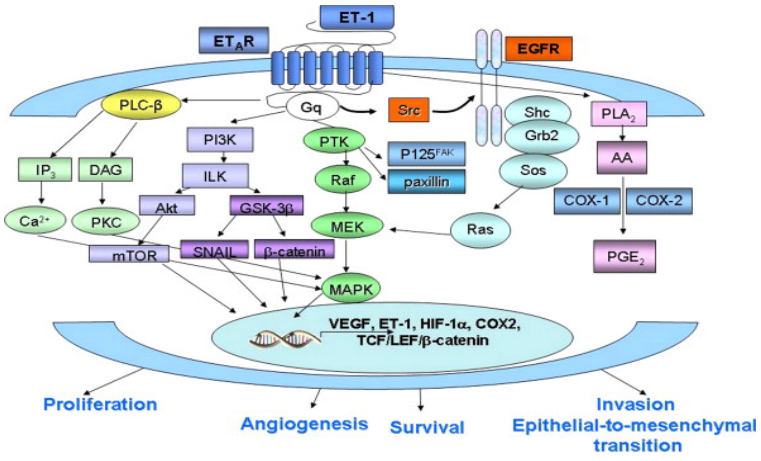

Figure 2: Molecular mechanisms of endothelin axis in cancer cell, Bagnato A et al. 2008.

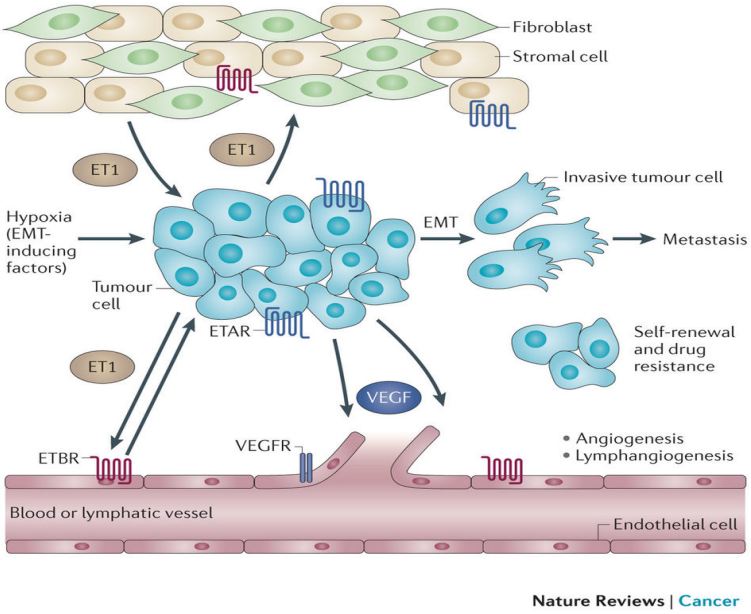

Figure 3: Role of endothelin in angiogenesis in human cell, Rosanò L et al. 2013.

the importance of the ET-1 axis in cell survival. Previous studies have indicated a potential function for ET-1 in cell proliferation or as a cell survival factor [48]. The binding of ET-1 to high-affinity sites inhibits FasL-induced apoptosis, while the binding of either ET-1 or receptor antagonists to low-affinity sites promotes FasL-induced apoptosis. Thus, ET signaling pathways do not induce human cancer cell proliferation, but are survival signals controlling resistance to apoptosis [51].

Endothelin Receptor Antagonists Drugs

Prospective clinical randomized controlled trials (RCTs) have demonstrated the efficacy and safety of endothelin receptor antagonist in different time for different indication. Currently, there are three endothelin receptor antagonists approved for the treatment of pulmonary artery hypertension. Bosentan, Ambrisentan, and Macitentan are the three drugs approved and widely used for treatment of PAH [46]. Before Ambrisentan, sitaxsentan (Thelin) was the first selective ETA receptor antagonist made available by the European Regulatory Agency, in 2006 (52). Multi-center, randomized, placebo-controlled clinical trials have demonstrated that sitaxsentan has beneficial effects on exercise capacity (i.e., 6-min walk distance [6MWD]), functional class (FC), and hemodynamic parameters in PAH patients. However, cases of fatal liver toxicity led the European Medicines Agency (EMA) to withdraw marketing authorization for sitaxsentan in 2010 [20].

Bosentan: Bosentan is a non-peptide pyrimidine derivative that competitively antagonizes the binding of ET-1 to both ETA and ETB receptor subtypes and irreversibly blocks their activities. It was developed in 1991 [52]. Bosentan has a specific inhibition of ET-1 receptors, with non-binding to other receptors, and was the first ERA studied and approved in PAH [46]. It was the first ERA and first oral medication approved for use in PAH. Its availability represents major progress in the management of the disease by improving clinical status, exercise capacity, and hemodynamic parameters, and delaying clinical worsening of PAH [53]. Bosentan, like the other ERAs, is an oral medication. The usual dosage is 125 mg twice daily after a titration period of 4 weeks (62.5 mg twice daily). It is also available for children in a dispersible tablet formulation (32 mg) that has the same pharmacokinetic properties as the adult formulation [53]. This formulation can also be used in adults with swallowing disorders. The pharmacokinetics of bosentan has mainly been studied in healthy populations. Data obtained from PAH patients indicate that exposure to bosentan is about twofold higher than in healthy populations, whereas the pharmacokinetics of bosentan in pediatric PAH patients is comparable to that in healthy subjects [46]. Following oral administration, bosentan reaches peak plasma concentrations in healthy subjects after approximately 3–5 h. The absolute bioavailability is about 50 % and is not significantly modified with food at the recommended dosage of 125 mg. Bosentan is highly bound to albumin (around 98 %) and does not enter erythrocytes. No dosage adjustment in adults is required based on sex, age, ethnic origin, or bodyweight. Steady state concentrations are achieved within 3–5 days after multiple-dose administration, with a volume of distribution of 30 L and a clearance of 17 L/h [43]. The metabolism of bosentan is mainly hepatic and involves cytochrome P450 (CYP) 2C9 and CYP3A4, with three identified metabolites eliminated by biliary excretion. Among these metabolites, Ro 48-5033 is pharmacologically active and contributes to around 20 % of the total response following administration of bosentan[52]. Less than 3 % of the oral dose of bosentan is found in urine, therefore severe renal impairment (creatinine clearance 15–30 mL/min) has no clinically relevant influence on the pharmacokinetics of bosentan. No dose adjustment is required in mild hepatic impairment (Child-Pugh class A); however, moderate and severe hepatic impairment are contraindications for bosentan therapy [46]. Generally, multiple drug interactions with bosentan are reported due to its property of enzymatic induction of CYP2C9, CYP3A4, and probably also CYP2C19 and P-glycoprotein: bosentan decreases exposure to cyclosporine, glibenclamide, simvastatin, and warfarin by up to 50 % because of induction of CYP3A4 and/or CYP2C9.

Ambrisentan: Selective ETA receptor inhibition has theoretical benefits in terms of preserving vasodilator and clearance functions specific to ETB receptors, while preventing vasoconstriction and cellular proliferation mediated by ETA receptors [54]. It approved by the US FDA in 2007 and by the EMA in 2008, it is the only selective ERA available for the treatment of PAH [55]. Unlike bosentan (sulfonamide ERA), ambrisentan belongs to the carboxylic ERA group [46]. After oral administration (5 or 10 mg once daily),

ambrisentan is rapidly absorbed into the systemic circulation with a bioavailability of about 90 % [43]. Food has no impact on this bioavailability [46]. It is highly bound to albumin in the same range as bosentan and is sparsely distributed in erythrocytes. Steady state is obtained after 4 days of treatment. The main metabolic pathways of ambrisentan are glucuronidation (13 %), oxidation by CYP3A4 (and to a lesser extent CYP3A5 and CYP2C19), leading to 4-hydroxymethyl ambrisentan (21 %). Affinity of this metabolite on ETA receptors is 65 % less than that of ambrisentan and is not part of the pharmacologic activity of the drug. Due to metabolization, treatment with ambrisentan should be avoided in patients with severe hepatic impairment. Both biliary (around 80 %) and urinary (around 20 %) routes are involved in ambrisentan excretion [43, 46]. Unlike bosentan, ambrisentan has a low potential for drug-drug interactions, explained by the small effect on hepatic CYP450 induction or inhibition [56]. It can be safely administered with warfarin or sildenafil without dose adjustment (57). Similarly, no relevant pharmacokinetic changes were detected with combined administration of ethinyl estradiol/norethindrone and ambrisentan, leading to no requirement for dose adjustment [56].Significant interaction was only reported with cyclosporine A, with a twofold increase in ambrisentan concentration leading to fixed dose adjustment at 5 mg daily [57]. Unlike other ERAs, no experimental data are available on the effects of ambrisentan in pulmonary hypertension. In terms of inflammation, treatment with ambrisentan decreases expression of pro-inflammatory genes in ischemia/reperfusion models, leading to a cytoprotective effect on vascular microcirculation [58].

Macitentan: Macitentan is a new potent non-peptide non-selective ERA with a 50-fold higher affinity for ETA than for ETB receptors. Development of macitentan led to a high level of tissue targeting and sustained receptor binding compared with other ERAs. The FDA (October 2013) and the EMA (December 2013) approved macitentan for the long-term treatment of PAH as monotherapy or in combination in adult patients [46]. The pharmacokinetics of macitentan is dose proportional and characterized by slow absorption due to low aqueous solubility. At a dose of 300 mg, macitentan has a median time to Cmax (tmax) of about 8 h and a half-life of 17.5 h, compatible with a once-daily dosing regimen [59]. In vivo, macitentan is metabolized into a major and pharmacologically active metabolite, ACT-132577, which is formed by oxidative depropylation through CYP3A4. While ACT-132577 is fivefold less potent than macitentan, its long half-life (about 48 h) leaves it prone to accumulate upon repeated dosing and therefore significantly contributes to the overall effect [46]. Urinary excretion is the most important route of elimination of drug-related material compared with feces in humans. In urine, four entities were identified, with the hydrolysis product of ACT-373898 the most abundant. In feces, five entities were identified, with the hydrolysis product of macitentan and ACT-132577 the most abundant. Report showed that no clinical relevance and no requirement for dose adjustment in renal and hepatic impairment treated with macitentan [60]. Macitentan is a competitive ERA with significantly slower receptor dissociation kinetics than the other approved ERAs. Slow dissociation caused insurmountable antagonism in functional pulmonary arterial SMC-based assays; this could contribute to an enhanced pharmacological activity of macitentan in PAH. In functional assays, macitentan and its metabolite inhibited ET-1-induced contractions in isolated endothelium-denuded rat aorta (ETA receptors) and sarafotoxin S6c-induced contractions in isolated rat trachea (ETB receptors). In pulmonary hypertension rats, macitentan prevented both increases in pulmonary pressures and RVH and improved survival without any effect on systemic arterial blood pressure [61].

Enrasentan: It is a mixed ETA and ETB receptor antagonist with a higher affinity for ETA receptors, although it cannot be considered a selective antagonist. It is (+)-(1S,2R,3S)-3-(2-(2-hydroxyeth-1-yloxy)-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5(prop1yloxy)indan-2-carboxylic acid [42]. The bioavailability of the drug was calculated as dose-normalized AUC (intraduodenal) dose-normalized AUC (intravenous) and the result was 66%. The terminal half-life was 3.3 h; it was calculated by least-squares linear regression analysis of the log-transformed concentration-time data [62]. The drug produced a concentration- dependent displacement of radioiodine labeled ET-1 in both receptor subtypes with Ki values of 1.1 and 111 nM for ETA and ETB receptors, respectively. In human isolated pulmonary artery enrasentan produced a 7-fold shift to the right of the ET-1 concentration-response curve, with a calculated Kb value of 5.2 nM[42].

Potential clinical application of Enrasentan includes: Heart Failure; Elevated plasma levels of endothelins in patients with heart failure. The levels of endothelins are positively correlated with the NYHA functional class and negatively with the ejection fraction. High levels of plasma endothelins are a negative prognostic marker in patients with heart failure [62]. These peptides exert several activities with potential negative effects on the heart. They augment sympathetic tone, increase sodium retention and the activity of the renin-angiotensin system. They also increase cardiac inotropism, have proarrhythmogenic effects and induce deposition of matrix proteins [63]. The drug prevented the progressive deterioration of cardiac output; cardiac index and stroke volume observed in the control group and attenuated left ventricle hypertrophy induced by high blood pressure. Plasma levels of aldosterone and ANF, recognized markers of cardiac dysfunction, were significantly reduced in rats treated with enrasentan. It also reduced the overall mortality with heart failure by 65% when it gives with others conventional heart failure drugs [64]. Pulmonary Arterial Hypertension; ET-1 is overexpressed in the plasma and lung tissue of patients with pulmonary arterial hypertension (either primary or associated with scleroderma) [65]. This peptide not only has a very powerful vasoconstrictor activity in the pulmonary vascular bed but it can also induce smooth muscle proliferation in the vessel wall [66]. The effects of enrasentan on pulmonary hypertension have been evaluated in vitro and in vivo. In vitro enrasentan prevented the ET-1-induced contractions of isolated pulmonary artery rings from guinea pigs. In animals treated with enrasentan pulmonary artery pressure and right ventricle hypertrophy were significantly attenuated, while the drug had no effect on the erythropoietic response to hypoxia [65]. Renal Protection; ET-1 transgenic mice develop interstitial renal fibrosis and glomerulosclerosis with progressive heart failure, although blood pressure is not affected by transgene expression. ET-1 is not only a potent vasoconstrictor agent in the kidney but is also known to trigger mesangial cell constriction, to enhance glomerular cell proliferation and to stimulate extracellular matrix accumulation [64]. At low dose enrasentan (30 micgm/kg/min)to instrumented dogs. They showed that the drug not only prevented the decrease in renal plasma flow, urine flow and sodium excretion but also reversed these effects. At a higher dose of enrasentan(100 micgm/kg/min) the effects of endothelins were prevented but no renal vasodilation or natriuresis was observed. At low dose ETA but not ETB, are blocked so that ET-1 infusion increases renal blood

flow and sodium excretion by stimulating the latter receptors [67]. It is very promising. However, clinical studies are necessary to confirm a possible role for these drugs in the prevention and treatment of kidney disease [42]. Arterial Hypertension; ET-1 is one of the most potent vasoconstrictors but it is also a trophic factor and a mitogen. These two additional properties are of interest considering the trend to improve end organ remodeling in hypertensive patients in addition to normalizing blood pressure. For these reasons it has been suggested that endothelin receptor antagonists could play an important role as antihypertensive agents even though there is no convincing evidence that endothelins are involved in the pathogenesis of essential hypertension [42]. The antihypertensive effect of enrasentan has been evaluated in a few studies. Cosenzi et al. have demonstrated that the drug normalizes blood pressure and protects target organs in rats with hypertension induced by a high fructose diet [67]. Stroke; Many authors have demonstrated the increased plasma level of endothelins in patients with ischemic stroke, as compared to healthy controls. ETA receptors are responsible for vasospasm; this suggested that for the protection of neuronal cells from ischemic injury a dual blockade of ET receptors might be preferable. No data on the efficacy of endothelin antagonists in patients with strokes are available [64].

Tezosentan: It acts as a vasodilator and was designed as a therapy for patients with acute heart failure. Recent studies have shown however, that tezosentan does not improve dyspnea or reduce the risk of fatal or nonfatal cardiovascular events. It is a non-selective ETA and ETB receptor antagonist [68]. Tezosentan is an IV competitive antagonist of endothelin 1 on both endothelin A and endothelin B receptors [67] and preclinical studies have demonstrated that tezosentan improves hemodynamics and renal function in rats with heart failure and decreases pulmonary edema and improves survival in rats with AHF due to myocardial infarction [69]. In clinical studies, plasma concentrations of endothelin 1 predict the occurrence of ventricular arrhythmias, recurrent heart failure events and mortality in patients with AHF. Thus, it is reasonable to anticipate that endothelin receptor antagonists would have favorable clinical effects in the treatment of acute heart failure [67]. Intravenous (i.v.) injection of tezosentan is effective in animal models of hypertension, acute renal failure and heart failure in which it increases cardiac output and renal blood flow and decreases peripheral and pulmonary pressures, pulmonary edema and induces coronary vasodilatation [67]. It's elimination half-life of approximately 3 hours. In vivo metabolism of tezosentan in rats is very limited. The predominant elimination mechanism is biliary excretion of unchanged compound. Preliminary data indicate that in humans tezosentan is also for the major part excreted unchanged into faeces whereas unchanged tezosentan in urine accounts for approximately 2% of the administered dose [70]. Plasma concentrations reached steady-state conditions within a short period of time [48].

Sitaxentan: Sitaxsentan sodium is a selective ETA receptor antagonist, and is chemically designated as molecular formula of C18H14ClN2NaO6S2 (71). ET-1 actions are mediated through endothelin ETA receptors, present on pulmonary vascular smooth muscle cells, ETB receptors, present on pulmonary vascular endothelial cells and, to a lesser extent, on pulmonary vascular smooth muscle cells. Sitaxsentan is a selective ETA receptor antagonist with a high oral bioavailability (~ 90%), and a long duration of action (with a half-life of ~ 10 h in PAH patients) [71]. Sitaxsentan is rapidly absorbed after oral administration, reaching maximum plasma concentrations in PAH patients within 1–4 h. The terminal elimination half-life (t½) is 10 h [71]. Following oral administration to healthy volunteers, sitaxsentan is highly metabolized. The most common metabolic products are at least 20-times less potent as ETA receptor antagonists than sitaxsentan in a standard in vitro test of activity. In vitro, sitaxsentan is metabolized by CYP2C9 and CYP3A4; however, administration of sitaxsentan with inhibitors of these isoforms has not resulted in clinically significant changes in sitaxsentan plasma concentrations [72]. Of any oral dose that is administered 50-60% is excreted in the urine, with the remainder being eliminated in the feces; and < 1% of the dose is excreted as unchanged drug [71]. Its absolute bioavailability of sitaxsentan is estimated to be 89%, and is unaffected by food. Sitaxsentan is more than 99% protein bound to plasma proteins, predominantly albumin. The degree of binding is independent of concentration in the clinically relevant range. Sitaxsentan does notpenetrate into erythrocytes and does not seem to cross the blood–brain barrier and drug [71].

ET Receptor Antagonists On Pipeline

For Cancer: ET-1 has shown pleiotropic mechanisms in different pathways of cancer development and progression. For this reason, many researchers have hypothesized a role of the ET-1 axis targeting for cancer treatment [48]. Various approaches have been identified in order to impair ET biological function in tumors, for example, inhibition of ET biosynthesis, blockade of ET production from prepro ETs, promotion of ET degradation, selective blockade of ETAR/ETBR activation and enhancement of tumor perfusion by ETBR activation to potentiate efficacy of antineoplastic drugs. Among all these options, the ET receptor blockade has reached the most advanced phases of preclinical and clinical drug development. Several small molecules functioning as ETAR antagonists have provided a clearer understanding of the physiologic role of ET-1 and its effects on ET receptormediated signal transduction in tumor development and progression. Atrasentan and ZD-4054 are the most potent and selective ETAR antagonists. They are orally bioavailable. For this reason, they are well qualified for clinical development in cancer treatment [48, 73].

Atrasentan is able to inhibit effectively cell proliferation and VEGF secretion both in ovarian carcinoma cell lines and primary cultures. This action of atrasentan can be translated into biological effects such as reduction of microvessel density, VEGF and MMP-2 expression and increase in the percentage of apoptotic tumor cells [71]. Atrasentan has induced tumor growth inhibition in xenografts of cervical carcinoma cells. This effect has been observed with just two cycles of treatment. It has promising outcome in phase one and phase two clinical trial for the treatment of cervical carcinoma, prostate cancer, nasopharyngeal and bladder cancers [72].

Zibotentan (ZD-4054) is an orally active specific ETAR antagonist. ETAR blockade by zibotentan inhibits ET-1-induced mitogenic effects, while the ETBR antagonist. It has promising in results in preclinical trial for treatments of ovarian carcinoma cells [74].

A-182086 is another dual antagonist. It can inhibit tumor growth by blocking proliferation not only of tumor cells but also of endothelial cells expressing ETBR. It has shown a potential role in Kaposi sarcoma treatment because of simultaneous interference with cell proliferation, invasiveness and angiogenesis [73].

BQ788 is a peptide ETBR antagonist which inhibits growth and induced death of melanoma cells in vitro and in vivo. A-192621 is a specific ETBR antagonist studied in xenografts of human

melanoma cells. It suppresses HIF-1a accumulation, tumor growth, neovascularization and VEGF and MMP-2 expression [73].

For cardiovascular disorder: The ETA-selective, peptide antagonist BQ-123 was the first agent to be widely available to investigate the role of ET in heart failure [40]. BQ-123 treatment of rats with chronic myocardial infarction due to coronary artery ligation resulted in significant reductions in LV hypertrophy and chamber enlargement and also increased survival [48]. Bosentan is an oral non-peptide, dual ERA, which has been demonstrated to have beneficial effects in multiple models of heart failure, including the rat coronary artery ligation. Currently, it has promising effects under per-clinical phase one investigation [73]. BQ-123, darusentan (LU135252), sitaxsentan (TBC 11251), BMS-193884, and ABT-627 have significant acute hemodynamic effects in patients with cardiovascular disorder. Currently they are under clinical trials [40].

CONCLUSION

Endothelin is synthesized in vascular endothelial and smooth muscle cells, as well as in neural, renal, pulmonic, and inflammatory cells. It consists of four closely related peptides, endothelin (ET)-1, ET-2, ET-3, and ET-4. These peptides are converted by endothelin-converting enzymes (ECE-1 and -2) from 'big endothelins' originating from large pre-pro-endothelin peptides cleaved by endopeptidases. ET-1 is the principal isoform in the human cardiovascular system and remains the most potent and unusually long-lasting constrictor of human vessels so far discovered. In the endothelium, ET-1 is released abluminally toward the vascular smooth muscle, suggesting a paracrine role. It acts through a seven transmembraneendothelin receptor A (ETA) and endothelin receptor B (ETB) receptors belongs to G protein-coupled rhodopsin-type receptor superfamily. The cellular effects of ET, including constriction of vascular smooth muscle, cell proliferation, hypertrophy of cardiac myocytes, and activation of cardiac fibroblasts, are those associated with both the clinical manifestations of heart failure, pathologic remodeling of the heart that results in progressive cardiac dysfunction and excessive cell proliferation. This peptide involved in pathogenesis of cardiovascular disorder like (heart failure, arterial hypertension, myocardial infraction and atherosclerosis), renal failure, pulmonary arterial hypertension and it also involved in pathogenesis of cancer. Potentially endothelin receptor antagonist helps the treatment of the above disorder. Currently, there are a lot of trails both per-clinical and clinical on endothelin antagonist for various cardiovascular, pulmonary and cancer disorder. Some are approved by FAD for the treatment. These agents are including both selective and non-selective endothelin receptor antagonist (ETA/B). Currently, Bosentan, Ambrisentan, and Macitentan approved for the treatment of pulmonary arterial hypertension. The aim of this seminar is briefly introduce endothelin system and its antagonist drugs with their detail pharmacological and

COMPETING INTERESTS

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All authors were involved in the design, write up and preparing the manuscript to be submitted. All authors have read and agreed the manuscript.

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