Endothelin receptor antagonism during preeclampsia: a matter of timing?

Hitzerd E, Neuman RI, Mirabito Colafella KM, Reiss IKM, van den Meiracker AH, Danser AHJ, Visser W, Versmissen J, Saleh L

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ABSTRACT

Preeclampsia (PE) is a pregnancy complication, featuring elevated blood pressure and proteinuria, with no appropriate treatment. Activation of the endothelin system has emerged as an important pathway in PE pathophysiology based on experimental PE models where endothelin receptor antagonists (ERAs) prevented or attenuated hypertension and proteinuria. Hence, ERAs have been suggested as potential therapy for PE. However, developmental toxicity studies in animals have shown severe teratogenic effects of ERAs, particularly craniofacial malformations. Nonetheless, sporadic cases of pregnancy in women using ERAs to treat pulmonary hypertension have been described. In this review we give an overview of cases describing ERA use in pregnancy and critically address their possible teratogenic effects. A systematic search in literature yielded 18 articles describing 39 cases with ERA exposure during human pregnancy. In most cases there was only exposure in the first trimester, but exposure later or throughout pregnancy was reported in 5 cases. Elective termination of pregnancy was performed in 12 pregnancies (31%), two ended in a spontaneous miscarriage (5%) and no fetal congenital abnormalities have been described in the remaining cases. These preliminary findings support the idea that ERA treatment for severe, early onset PE might be an option if applied later in pregnancy, when organogenesis is completed to avoid teratogenic risks. However, third trimester toxicology studies are warranted to evaluate drug safety. Subsequently, it remains to be established whether ERA treatment is effective for alleviating maternal symptoms, as demonstrated in preclinical PE models, allowing pregnancy prolongation without leading to adverse neonatal outcomes.

INTRODUCTION

Preeclampsia (PE), a syndrome featuring de novo hypertension accompanied by proteinuria and/or evidence of maternal acute kidney injury, liver dysfunction, neurological features, hemolysis or thrombocytopenia, and/or fetal growth restriction (FGR), is the most frequently encountered medical complication during pregnancy.[1] Ultimately, PE may lead to severe complications, thereby increasing maternal, fetal and neonatal morbidity and mortality.[2, 3] While randomized trials have tested interventions with different antihypertensive agents including methyldopa and various calcium antagonists, these drugs only show a temporary effect on stabilization of the clinical manifestations of PE, but not on hard clinical outcomes such as mortality.[4-6] To date, the only effective treatment of PE is delivery of the placenta and hence the baby, often severely premature. Consequently, novel treatment strategies to prevent or alleviate PE are urgently needed. Achieving an additional week of gestational age in premature infants enhances fetal maturity and hereby leads to a decrease in fetal mortality and enhances neonatal outcome.[7] There is strong experimental evidence that activation of the endothelin (ET)-system plays a key role in the pathophysiology of PE.[8] In addition, increased ET-1 levels have been reported in PE compared to healthy pregnancies.[9] A possible therapeutic strategy could therefore be to target the activated ET-axis by using endothelin receptor antagonists (ERAs).[8] However, the latter approach remains controversial since developmental toxicity studies have shown serious teratogenic effects in offspring of animals treated with ERAs during pregnancy. Nonetheless, it is important to note that this teratogenicity observed in animals does not necessarily translate to humans. The aim of this review is to provide a complete overview of ERA use in pregnancy, based on evidence from humans and animals, and to critically address their potential teratogenic effects.

ENDOTHELIN-1 AS THE ROOT CAUSE OF PREECLAMPSIA

Though not fully elucidated, evidence suggests that the activation of the ET-axis is strongly linked to the manifestations of PE.[10, 11] The ET-system consists of a family of 3 structurally closely related amino acid peptides (ET-1, ET-2 and ET-3). The main ET synthesized and secreted by endothelial cells is ET-1, this peptide is also synthesized and secreted by the syncytiotrophoblasts of the placenta.[12] ET-1 exerts its effect by binding to the cell-membrane G-protein-coupled ET type A receptors (ET_AR) and ET type B receptors (ET_BR).[13] Activation of ET_AR and ET_BR on vascular smooth muscle cells initiates prolonged vasoconstriction as well as cell proliferation. Conversely activation of the ET_BR on endothelial cells mediates vasodilation by releasing nitric oxide (NO) and prostacyclin (Figure 1). ET_BR activation may therefore counterbalance the ET_AR-mediated

vasoconstrictive response.[14, 15] In addition, the ET_BR serves as a clearance receptor for endothelins.[16]

ET-1 in pregnancy is regulated by numerous factors as recently reviewed by Granger *et al*.[8] The functional role of the ET-system in healthy pregnant women is not well known, it seems to promote first trimester trophoblast proliferation and invasion,[17] and an increased ratio of ET_BR to ET_AR has been observed in human placental villi during their development (Figure 1).[18] Furthermore, while maternal serum levels of ET-1 during healthy gestation remain similar to those of non-pregnant women, a substantial rise is detected during labor,[19, 20] implicating ET-1 involvement in the initiation of uterine contractions.[21] In addition, high concentrations of ET-1 in the amniotic fluid and in the fetal circulation are observed, being almost 3-fold higher in the umbilical vein when compared to maternal serum levels around labor.[22]

In animal models inducing placental ischemia, chronic elevation of soluble Fms-like tyrosine kinase-1 (sFlt-1), infusion of tumor necrosis factor-alpha (TNF-α) or agonistic autoantibodies to the angiotensin II type I receptor (AT1-AA), all induce a PE-like syndrome. In all these animals a significant increase in the expression of the precursor peptide prepro-ET-1 is seen in the kidney, as well as in the maternal vasculature in the case of AT1-AA and in the case of TNF-α in both the maternal vasculature and the placenta.[23-25] This enhanced prepro-ET-1 expression likely translates in higher ET-1 levels, underlying the rise in blood pressure in PE, as this rise can be prevented by ERAs. Furthermore, in these animals the ET_BR is downregulated in endothelial cells.[26] Similarly, decreased ET_BR expression in vascular endothelial cells has been found in pregnant women suffering from PE.[27] Subsequently, a wide range of clinical studies in pregnant women has observed a 2- to 3-fold rise of circulating ET-1 in PE compared to normal pregnancies.[9, 10, 28, 29] In addition, multiple regression analysis revealed that ET-1 is an independent determinant of urinary protein in PE and of the suppression of the renin-angiotensin aldosterone system in this condition.[10]

Regarding ERA effects in the treatment of the maternal syndrome of PE, knowledge comes from experimental animal models of PE. For example, in the reduced uterine perfusion pressure model in rats, ET_AR blockade reduced maternal blood pressure.[30] This is suggestive for an upregulation of the ET-1-ET_AR pathway in this model, which when blocked allows counter regulatory factors (like NO) to lower blood pressure.[8, 31] In rats infused with sFlt-1 and soluble endoglin, treatment with selective ET_AR antagonists also significantly decreased hypertension, urinary protein-to-creatinine ratio, hemolysis and liver enzymes, and increased platelets (Table S1, online-only data supplement).[32] Moreover, compared to placebo, FR-139317, an ET_AR antagonist (Table 1), potentially by improving placental perfusion, prevented FGR in rats placed in a hypoxic environment (Table S1).[33]

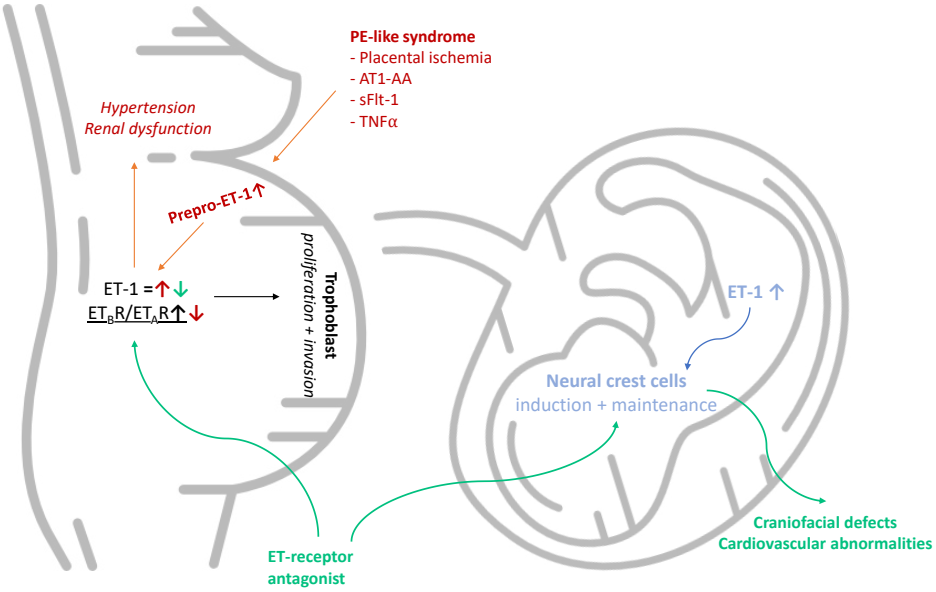

Figure 1. Role of endothelin in healthy pregnancies and preeclampsia-like syndrome. This figure shows the ET-1 levels in healthy pregnancy and non-pregnant women (in black) and the effects of a PE-like syndrome (in red). In blue the effects of increased ET-1 levels in the fetal circulation during healthy pregnancy are shown and in green the effects of ERAs on ET-1 levels.

Table 1. Different endothelin receptor antagonists and their targeted receptor.

ERA	Receptor blockade
A-127722	ET_A
A-182086	ET_A / ET_B
ABT-546	ET_A
ABT-627	ET_A
Ambrisentan	ET_A
Bosentan	ET_A / ET_B
BQ-123	ET_A
BQ-788	ET_B
FR-139317	ET_A
L-753,037	ET_A
SB-209670	ET_A / ET_B
SB-217242	ET_A / ET_B
Sitaxentan	ET_A
TBC-3214	ET_A

Thaete *et al*. examined the effect of selective ET_AR (A-127722 and FR-139317) and dual (A-182086) ERAs on fetal and placental growth in rats, with and without long-term NO synthase (NOS) inhibition with L-NAME. The dual ERA resulted in fetal and placental growth restriction, without attenuating the fetal and placental growth restriction caused by L-NAME infusion. In contrast, the selective ET_AR antagonists improved fetal and placental growth during NOS inhibition, and were without adverse effects in the absence of NOS inhibition.[34] These studies suggest that blocking ET_AR-mediated ET-1 signaling might be more beneficial in PE or FGR then dual ET_AR/ET_BR blockade. Selective ET_BR blockade in the same PE animal model with L-NAME was lethal for all animals.[35]

Nonetheless, excitement about ERAs as a possible treatment for PE has been dampened by severe teratogenic effects following maternal ERA administration as well as by ET-1, ET-3 and ET_AR or ET_BR gene disruption experiments during gestation in animal models, in particular serious craniofacial and cardiovascular malformations, pigmentary abnormalities and aganglionic megacolon.[36] Because of this, clinical trials studying ERA treatment to alleviate or even to prevent PE are lacking.

ENDOTHELIN-1 DURING EMBRYOGENESIS

To explain the occurrence of teratogenic effects during ERA treatment, understanding the role of ET-1 during embryogenesis is crucial. During early embryonal development, the ET-pathway plays an important role in the induction and maintenance of neural crest cells,[37] cells migrating to many different locations and differentiating into a wide variety of cell types including the craniofacial skeleton, cartilage, neurons and glia of the peripheral nervous system, connective tissue, neuroendocrine cells, and melanocytes.[38] The ET-axis is also involved in maintaining the high vascular resistance that is needed in the fetal lung development during pregnancy.[39] In the lung ET_AR is abundantly expressed during both the pre- and (early) postnatal periods, whereas the ET_BR is mildly expressed during early lung development. The lung ET_BR expression increases and stabilizes in the last prenatal stages and after birth, preventing muscularization of pulmonary pre-capillaries by stimulating vasodilation and ET-1 clearance.[40] Alteration of ET-1 or its receptors seems to affect normal embryonal development by impairing neural crest cell migration,[36, 41] as has been demonstrated by studies using murine knockout models.[42, 43] Clouthier *et al*. showed that ET_AR deficient mice have severe craniofacial defects, such as underdeveloped mandibles and abnormal middle ear structures. If left untreated, pups died shortly after birth because of asphyxia due to these structural defects. Furthermore, there was a 100% cumulative penetrance of cardiovascular abnormalities, for example interruption of the aorta (44%), tubular hypoplasia (56%) and ventricular septal defect (92%).[42] ET-1 deficient mice also display cardiovascular malformations including

ventricular septal defect, absent right subclavian artery and interruption of the aorta. Importantly, the frequency and severity of these abnormalities increased when they were additionally treated with BQ-123, a selective ET_AR antagonist (Table S1). This suggests that the lack of ET_AR stimulation by ET-1 is compensated, at least in part, by other ET isoforms (e.g., ET-2, ET-3).[43]

It has also been suggested that ET-1 plays a key role in closure of the ductus arteriosus after birth, although evidence is conflicting. The ductus arteriosus is a shunt connecting the pulmonary artery and aortic arch in utero, this way most of the fetal blood bypasses the pulmonary circulation. At birth, blood oxygenation shifts from the placenta to the lungs, making the ductus arteriosus redundant and initiating its closure.[44] Some studies have indicated that oxygen-triggered ET-1 release regulates closure of the ductus through binding to ET_AR on vascular smooth muscle cells.[45, 46] However, other experimental studies have shown that although blocking the ET_AR indeed counteracts the constricting effects of ET-1, the ductus still closes, both *in vitro* and *in vivo*.[47, 48] Therefore, the exact effect of ET_AR blockade on closure of the ductus arteriosus remains uncertain.

TERATOGENIC EFFECTS OF ENDOTHELIN RECEPTOR ANTAGONISTS

With regard to their teratogenic effects, either selective or dual ERA exposure during animal pregnancy has supplemented the findings of gene disruption studies, revealing the crucial role of ET-1 in embryonal development.

Treatment of wild type 129S6 mice with an ET_AR antagonist (TBC-3214) on gestational day (GD) 8, 9 or 10 showed a significant increase in pups with craniofacial malformations compared to the control group (up to 100% depending on day of treatment). There were no differences in litter size, fetal weight or fetal mortality (Table S1).[49]

The toxicity of Sitaxentan on embryo-fetal development in Sprague-Dawley rats was evaluated by Cross *et al*.[45] Sitaxentan is a highly selective ET_AR antagonist (Table 1) that was withdrawn from therapeutic use in 2010 because of the risk of idiosyncratic liver injury. In this study, rats were divided into 3 groups of different treatment periods (GD 0-6, 6-16 or 16-lactation), and each group was subdivided to test multiple dosages ranging from 20-120 mg/kg/day. There were no differences in fetal weight and fetal mortality between treatment and vehicle groups. Treatment from GD 6-16 with a dosage of 80 mg/kg/day or higher resulted in a significant increase of craniofacial and cardiovascular malformations (Table S1).[50] Similarly, in another study Sprague-Dawley rats were treated with different dosages of another selective ET_AR antagonist (L-753,037) on GD 6-20. There were no differences in litter size and fetal mortality between treated and control groups. However, treatment with the dose of 40 mg/kg/day resulted in a significant reduction in fetal weight, and dosages of 10 mg/kg/day or higher resulted in an

increased incidence of congenital abnormalities (Table S1).[51] To further investigate the function of the ET-system in fetal development, Taniguchi *et al*. exposed pregnant rats to a selective ET_AR antagonist during 5 different gestational periods (GD 7-20, 7-9, 9-11, 11-13 or 7-8/11-20). Unfortunately, the ET_AR antagonist dosage was not specified, nor did the authors report litter size or fetal weight. However, the authors did observe that most congenital malformations were seen in offspring of rats that were treated on GD 7-20 or 9-11 (comparable to GD 13-64 and 17-22 in humans), with a 100% penetrance for craniofacial malformations. In the other treatment groups, no craniofacial malformations were seen (Table S1).[52]

Treinen *et al*. studied the effect of two dual ERAs (SB-217242 and SB-209670) on fetal development in Sprague-Dawley rats on GD 6-17. A significant decrease in litter size and fetal weight was observed at the highest dosages for each antagonist. Moreover, a dose-dependent increase in craniofacial and cardiovascular abnormalities was seen at doses of 50 mg/kg/day (SB-217242) and 10 mg/kg/day and higher (SB-209670). There was no difference in fetal mortality compared to control rats (Table S1).[53]

Lastly, the effect of different dosages of the dual ERAs SB-217242 and SB-209670 (administered on GD 6-20) on fetal development of New Zealand White rabbits has been studied. The SB-217242 treatment group showed a reduction in litter size at 50 mg/kg/day, while no effect was seen for treatment with SB-209670. For SB-217242, a dose-dependent increase in congenital abnormalities (predominantly craniofacial) was seen from 50 mg/kg/day, with 100% of fetuses being affected at 300 mg/kg/day. When rabbits were treated with SB-209670, an increase in congenital cardiovascular malformations was only seen at the highest dosage (Table S1).[53]

ENDOTHELIN RECEPTOR ANTAGONISTS IN HUMAN PREGNANCY

Currently, only the dual ERAs Macitentan, Ambrisentan and Bosentan are approved for clinical use for the treatment of pulmonary arterial hypertension (PAH) and digital ulcers due to systemic sclerosis not responsive to standard therapy. Beneficial effects of these drugs have also been shown in treatment of cancer and renal failure.[54] While ERA use is absolutely contraindicated during pregnancy, there are sporadic cases of women who used ERAs during a certain period of their pregnancy. We conducted a systematic search of the literature (Figure S1, online-only data supplement) up to 18 February 2019 to create an overview of cases reporting the use of ERAs in pregnancy (Table 2).

Thirty-nine cases of ERA use in human pregnancy have been described in 18 articles.[55-72] The study characteristics are provided in Table 2. All women were suffering from PAH, and many of them received combination treatment with e.g. sildenafil or prostacyclin-analogues. In 27 cases, the ERA used for treatment of PAH was the dual

Table 2. Overview of human cases in literature that reported use of ERAs in pregnancy.

Report	Country	N	ERA	Dosage	Treatment stopped (GA)	Outcome	Delivery (GA, weeks)	BW (g)	Perinatal outcome
Alvarez[55]	Argentina	1	bosentan	125 mg/bd	12 weeks	x	x	x	x
Bédard[56]	Multiple	1	bosentan	x	delivery	x	x	x	x
Cotrim[57]	Portugal	2	bosentan	62.5 mg/bd	delivery*	CS	29	x	healthy
			bosentan	125 mg/bd	1st trimester	TOP			
Daimon[58]	Japan	2	bosentan	250 mg/d	1st trimester	CS	29	1356	healthy
			ambrisentan	2.5 mg/d	15 weeks	CS	30	1298	IVH
Duarte[59]	USA	8	bosentan	x	1st trimester	CS	34	x	no CA
			bosentan	x	1st trimester	CS	36	x	no CA
			bosentan	x	1st trimester	CS	32	x	no CA
			bosentan	x	1st trimester	TOP			
			bosentan	x	1st trimester	TOP			
			bosentan	x	1st trimester	TOP			
			bosentan	x	1st trimester	TOP			
			bosentan	x	1st trimester	TOP			
Elliot[60]	UK	1	bosentan	x	6 weeks	CS	25	650	healthy
Jaïs[61]	Multiple	7	x	x	1st trimester	x	x	x	healthy
			x	x	1st trimester	x	x	x	healthy
			x	x	1st trimester	x	x	x	x
			x	x	1st trimester	TOP			
			x	x	1st trimester	TOP			
			x	x	1st trimester	TOP			
			x	x	1st trimester	TOP			

Table 2. Overview of human cases in literature that reported use of ERAs in pregnancy (continued).

Report	Country	N	ERA	Dosage	Treatment stopped (GA)	Outcome	Delivery (GA, weeks)	BW (g)	Perinatal outcome
Kaznica[62]	Poland	3	sitaxentan	x	1st trimester	miscarriage			
			bosentan	x	1st trimester	CS	33	x	no CA
			bosentan	x	1st trimester	CS	37	x	no CA
Kiely[63]	UK	2	bosentan	x	1st trimester	CS	26	650	no CA
			bosentan	x	1st trimester	CS	34	1580	no CA
Molelekwa[64]	Ireland	1	bosentan	x	28 weeks	x	30	1140	no CA
Price[65]	UK	1	bosentan	125 mg/bd	x	CS	28	x	x
Sahni[66]	USA	1	bosentan	x	1st trimester	CS	term	x	x
Smith[67]	USA	1	bosentan	x	4 weeks	CS	36	x	x
Sliwa[68]	Multiple	4	x	x	x	vaginal	term	x	healthy
			x	x	x	CS	preterm	SGA	healthy
			x	x	1st trimester	TOP			
			x	x	1st trimester	TOP			
Streit[69]	Switzerland	1	bosentan	125 mg/bd	5 weeks	CS	37	2760	healthy
Tokgöz[70]	Turkey	1	bosentan	125 mg/bd	delivery	CS	27	x	no CA
Verhaert[71]	USA	1	bosentan	x	1st trimester	miscarriage	x		
Zhang[72]	China	1	bosentan	x	delivery	CS	31	1420	healthy

*treatment started at 28 weeks gestation. Abbreviations: BW = birth weight; CA = congenital abnormalities; CS = cesarean section; ERA = endothelin receptor antagonist; GA=gestational age; IVH = intraventricular hemorrhage; SGA = small for gestational age; TOP = (medical) termination of pregnancy; x = data not available.

ERA Bosentan, while the ET_AR selective blockers Sitaxentan and Ambrisentan were each used in one case; the remaining 11 cases did not specify the type of ERA. ERA treatment was stopped in most patients early in the first trimester. In only 4 cases that continued pregnancy, the ERA was used throughout the entire pregnancy,[56, 70, 72] and in another case up to 28 weeks of gestation.[64] In the case described by Cotrim *et al*., there was only short exposure during the third trimester, as treatment with Bosentan was started at 28 weeks of gestation and the patient delivered one week later because of severe maternal deterioration related to PAH and premature rupture of membranes.[57] Three case reports did not provide information about the period of treatment.[65, 68] Only 8 case reports mentioned the ERA dosages that were used: 62.5-250 mg twice daily for Bosentan and 2.5 mg daily for Ambrisentan, [57, 58, 65, 69, 70] which are standard dosages for PAH treatment.[73] Elective termination of pregnancy was performed in 12 women (31%), all because of maternal condition related to PAH.[57, 59, 61, 68] Two patients had a spontaneous miscarriage (5%).[62, 71] Eighteen women delivered via caesarean section, one had a vaginal delivery and for 6 patients the mode of delivery was not reported. Gestational age at delivery ranged from 25 weeks to term. Most women delivered prematurely on maternal indication (worsening of PAH). In all 5 women that delivered at term treatment has been stopped in the first trimester. No congenital abnormalities were reported (Table 2).

Despite being absolutely contraindicated during pregnancy, the substantial number of reported cases of the use of ERAs during pregnancy is remarkable. Exacerbation of PAH symptoms is likely to occur in pregnancy because of pronounced pregnancy-induced hemodynamic changes, especially the rise in plasma volume and cardiac output.[74] From the reported cases leading to live offspring (n=25) no congenital abnormalities were reported, compared to a prevalence of 4% in the general population.[75] Also the percentage of miscarriages of 5% (2 of 39 reported cases) is lower than the percentage of 10-20% observed in the general population.[76, 77] It should be remarked that medically indicated elective termination of pregnancy was performed in a high number (31%) of women with PAH using ERAs, so it cannot be excluded that the number of miscarriages and congenital abnormalities has been underestimated. In the very limited number of women (n=5) who were exposed to Bosentan in the third trimester of pregnancy no congenital abnormalities were observed.

ENDOTHELIN RECEPTOR ANTAGONISTS AS TREATMENT FOR PREECLAMPSIA

Translating results from animal studies to humans remains difficult, especially since placental morphology differs greatly between species.[78] A method that allows to compare the development of the human embryo with that of for example rats is the Carnegie staging. These 23 stages are based on the morphological development of the vertebrate

embryo and can be applied to all vertebrates, given that the embryonic development of almost all vertebrates, including humans, is identical. As shown in Figure 2, in most of the animal studies that report severe congenital abnormalities ERAs were administered before the end of the Carnegie stages. In pregnant women, these drugs could be administrated later, e.g. at the end of the second trimester when organogenesis is complete and the symptoms of PE typically manifest. Our review of cases using Bosentan as treatment for PAH in women with term and preterm neonates showed no major (congenital) adverse effects as a consequence of treatment. This supports the idea that administration of ERAs later in pregnancy, i.e. from a gestational age of 20 weeks, could be safe. One should keep in mind that earlier administration might be more optimal as it may interfere with the origin of placental insufficiency leading to PE. However, since activation of the ET-axis appears to be involved in the clinical symptoms requiring preterm delivery, administration of ERAs at a later stage might be sufficient to delay delivery, thereby improving neonatal outcome. Another point of concern could be that, although organogenesis has been completed in third trimester of pregnancy, fetal lung maturation has not. Since the ET-axis is involved in fetal lung development[39] and ET_AR are abundantly expressed in the fetal lungs during the pre- and (early) postnatal periods,[40] ET_AR blockade at a later stage of pregnancy might counteract the high pulmonary vascular resistance needed for adequate lung maturation. Here it is important to realize that neonates born to mothers with PE have increased serum ET-1 levels compared to controls,[79] implying that ET_AR blockade might actually normalize this situation. Furthermore, neonates with PAH are often treated with the ERA Bosentan,[80, 81] after which no adverse effects of treatment on lung- and brain development have been described, although no long term follow-up studies are available.[80]

Knowledge about the placental transfer of ERAs is virtually non-existent. In rats, the fetal plasma level of the ERA ABT-546 was only 2% of the maternal plasma levels during maternal administration late in pregnancy, suggesting that only a minute fraction of the drug crosses the placenta.[82] Importantly, this study showed no adverse effect of ERA treatment late in pregnancy on pup weight or survival.[82] However, no third trimester toxicology studies have been performed, nor has any study assessed the transfer of ERAs in human placentas. With the *ex vivo* dual-sided placental perfusion model it is possible to obtain information about the transfer of different ERAs through the human placenta.[83] Although it seems likely that there will be (at least partial) placental transfer, given the low molecular weight and lipophilic properties of these drugs,[84] studying this will provide essential knowledge on placental pharmacokinetics. In addition, it can be examined whether the transfer is different in placentas derived from early onset PE pregnancies as compared to term or preterm uncomplicated pregnancies. Furthermore, methods preventing ERAs from passing the placental barrier (e.g. by linking them to

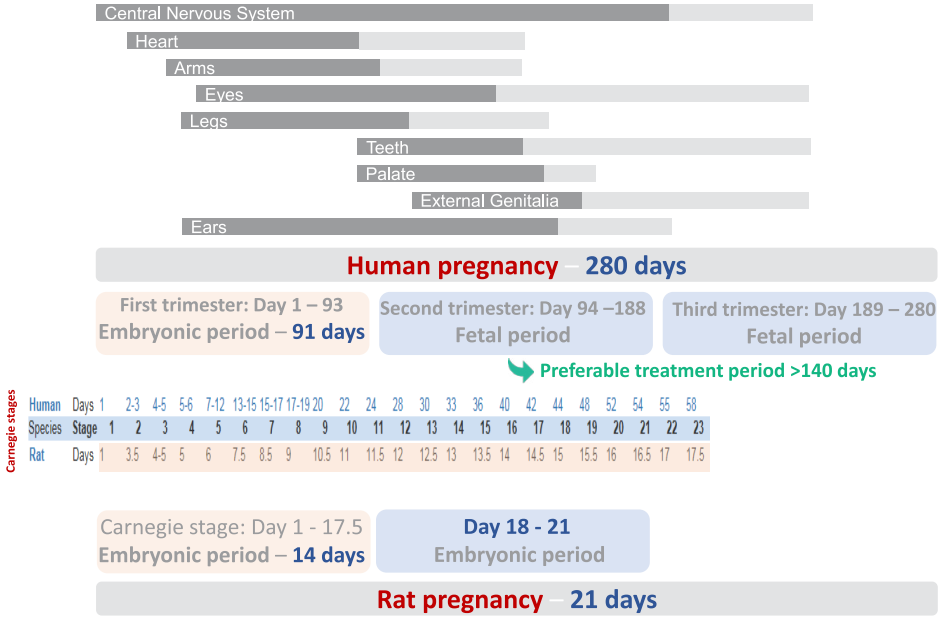

Figure 2. Developmental stages of pregnancy. This figure shows developmental stages of pregnancy in humans and rats, according to Carnegie staging as this can be applied to all vertebrates to compare the timing of development of different organs in different species. To reach the end of embryonic development (Carnegie stage 23) takes 17.5 days in rats and 58 days in humans. In pink highlighted, the period of ERA administration from day 1 – day 17.5, which is during all Carnegie stages in rat pregnancies as discussed in this paper. In green the time point when patients should be treated with ERAs is shown: 82 and 49 days after the Carnegie - and human embryonic stages respectively, much later when compared to the treatment period in animal studies.

elastin-like peptides) can be tested using this model. This approach would allow targeting the maternal ET-system without affecting the fetus.[8]

An important question would be whether a selective ET_A or a dual ERA would be most preferable. In different animal models of PE an ET_AR selective antagonist reverses symptoms without other adverse effects, supporting the use of an ET_AR selective receptor antagonist. In addition, selective inhibition/deletion of the ET_BR in animals is associated with distinct congenital abnormalities and death.[35, 85] On the other hand, based on the limited evidence in human pregnancies indicating that the dual ERA Bosentan, when administered in the third trimester of pregnancy in women with PAH, is not associated with craniofacial abnormalities would perhaps favor the use of this dual receptor blocker, especially because it is already approved for clinical use. A further possibility might be the application of endothelin-converting enzyme-1 (ECE-1) inhibitors, since ECE-1 activity is also increased in women with PE.[86] However, such drugs, by suppressing ET-1 formation, would mimic the effect of dual ERAs. Potential caveats are their selectivity and the possibility that ET-1 is also formed by non-ECE-1 enzymes, like chymase.[87]

Another important point of concern when considering ERAs for treatment of PE are their possible side effects. Some of the reported side effects are similar to PE symptoms, such as edema, headache, and elevated liver enzymes. When these PE symptoms occur during ERA treatment it will be very challenging to distinguish side effects from disease exacerbation. Another common side effect is anemia, and sometimes thrombocytopenia is seen. Therefore, plasma levels of hemoglobin, thrombocytes and liver enzymes should be closely monitored during treatment. Women with elevated liver enzymes, severe anemia or thrombocytopenia should not be started on ERA treatment. Furthermore, in case these side effects occur treatment might have to be discontinued in some cases.

CLINICAL IMPLICATIONS AND PERSPECTIVES

Given the increase in ET-1 during PE, targeting the ET-1 pathway in severe, early onset PE could potentially be a favorable new treatment strategy to improve maternal, fetal and neonatal outcomes when started after the completion of organogenesis. Especially since not all anti-hypertensive drugs can be used during pregnancy, due to either teratogenic effects or the lack of PE prevention. Recently, a randomized controlled trial studying the effects of sildenafil, a phosphodiesterase-5 inhibitor, versus placebo on pregnancy outcome in severe FGR has been halted, since sildenafil did not show beneficial effects and there were more neonatal complications in the treatment group in one of the cohorts.[88] Although no teratogenic effects of ERAs during late pregnancy have been reported in the limited number of human case reports, further research is needed to evaluate the pharmacokinetics and safety of these drugs in pregnancy, preferably by performing third trimester toxicology studies in animals with a longer gestation, like non-human primates. This is relevant because many of the previous experimental studies have used extremely high dosages, sometimes reaching 300 mg/kg/day, while patients with PAH are being treated with a maximum of 250 mg per day, i.e. 100 times less in a patient with an average weight of 70 kg. In addition, something that can be done relatively easy at this moment is to investigate the trans-placental transfer of the two clinically available ERAs that are FDA-approved, making use of the *ex vivo* human dual-sided perfusion model with cotyledons from both uncomplicated and PE pregnancies. Furthermore, the perfusion model might provide information on the passage (or lack thereof) of ERAs bound to peptides preventing them from crossing the placenta. At a later stage, we suggest that proof of principles studies should be performed in patients with very early onset (< 24 weeks of gestation) severe PE, in whom termination of pregnancy is considered, because of the severity of the maternal and/or fetal condition. In this setting, outcome parameters would be maternal blood pressure, proteinuria, and the disappearance of HELLP symptoms. In patients who are untreated we would

start with ERA, while in patients who already use antihypertensive treatment we would continue this medication and add an ERA, guided by the effect on blood pressure. In cases where blood pressure drops too much we would stop or decrease the already used antihypertensive medication.

In conclusion, the findings of this review support the idea that ERA treatment for severe early onset PE might be an option if applied later in pregnancy, when organogenesis is completed to avoid teratogenic risks, with close monitoring of closure of the ductus arteriosus. However, third trimester toxicology studies are warranted to evaluate drug safety and it remains to be established whether ERA treatment is effective for alleviating maternal symptoms, allowing pregnancy prolongation without leading to adverse neonatal outcomes.

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SUPPLEMENTAL INFORMATION

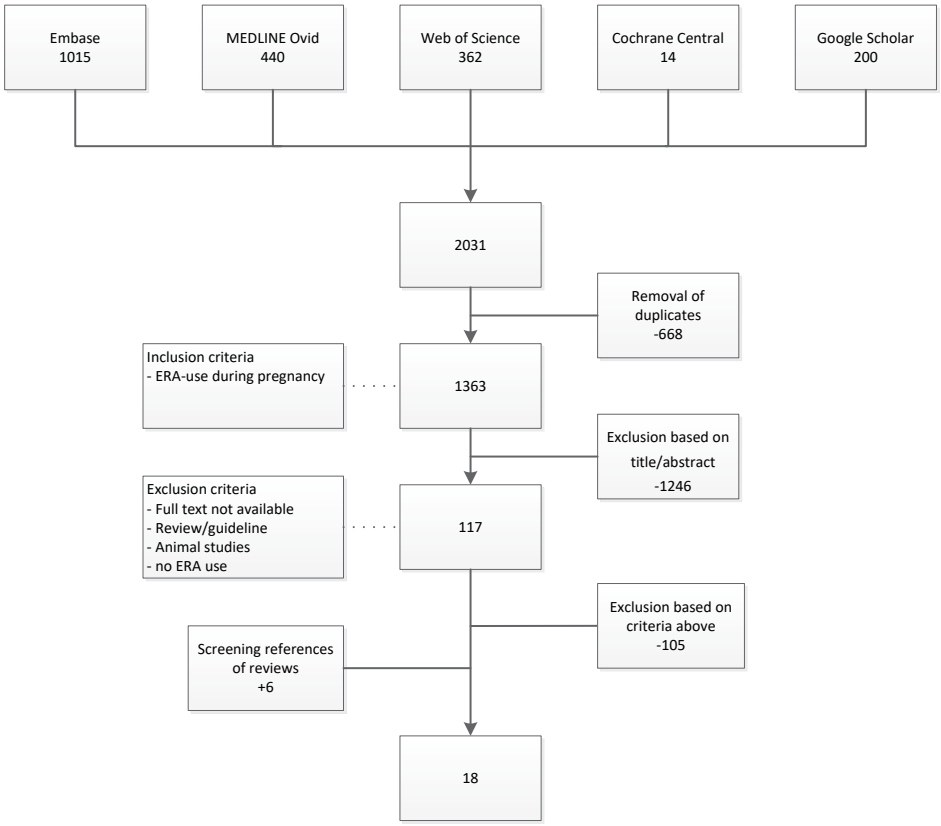

Figure S1. Flowchart of the systematic search in literature. A systematic search in five different databases was performed on 18 February 2019 and yielded 2031 articles. After de-duplication 1363 articles were left for screening. Based on title/abstract relevance 1246 articles were excluded and of the remaining 117 full text was screened. Finally, 18 articles describing 39 cases of ERA use during human pregnancy were found and included in this review.

Table S1. Overview of animal studies reporting administration of ERAs during pregnancy.

Study	Population	N	ERA	Dosage (mg/kg/day)	Treatment period (GD)	Sacrifice / delivery (GD)	Outcome*
				Reproductive toxicity			
Cross[50]	SD rats	24	sitaxentan	20/80/120	0-6	20	No differences in maternal weight gain, litter size, fetal- and placental weight, CA and fetal mortality
				Embryofetal developmental toxicity			
Cross[50]	SD rats	20	sitaxentan	20 bd/40 bd/80 bd/120	6-16	20	↓ Maternal weight gain at ≥ 40 mg ↑ CA (CF and CD) at ≥ 80 mg ND in litter size, fetal weight and mortality
Spence[51]	SD rats	25	L-753,037	2.5/5/10/20/40	6-20	21	↓ Maternal weight gain and fetal weight at 40 mg ↑ CA at ≥ 10 mg ND in litter size and fetal mortality
Taniguchi[52]	Rats	29	x	x	7-20	x	↑ CA (100% CF, 50% CD)
		18	x	x	7-9	x	no CA
		33	x	x	9-11	x	↑ CA (100% CF, no CD)
		33	x	x	11-13	x	↑ CA (no CF, 15% CD)
		20	x	x	7-8, 12-20	x	↑ CA (no CF, 25% CD), 100% mortality[†]
Treinen[53]	SD rats	10	SB-217242	0.01/1/10/50/300	6-17	21	↓ Maternal weight gain, litter size and fetal weight at 300 mg ↑ CA at ≥ 50 mg ND in fetal mortality
		10	SB-209670	0.01/1/10/50	6-17	21	↓ Litter size and fetal weight at 50 mg ↑ CA at ≥ 10 mg ND in maternal weight gain and fetal mortality

Table S1. Overview of animal studies reporting administration of ERAs during pregnancy (continued).

Study	Population	N	ERA	Dosage (mg/kg/day)	Treatment period (GD)	Sacrifice / delivery (GD)	Outcome*
	NZ white rabbits	8	SB-217242	0.01/1/10/50	6-20	29	↓ Litter size and fetal weight at 50 mg ↑ CA at ≥ 10 mg ND in maternal weight gain and fetal mortality
		8	SB-209670	0.01/1/10/25	6-20	29	↑ CA at 25 mg ND in maternal weight gain, litter size, fetal weight and fetal mortality
				Late gestation and lactation toxicity			
Cross[50]	SD rats	24	sitaxentan	20 bd/40 bd/60 bd	16-lactation	x	↑ CA at ≥ 40 mg bd (enlarged liver and lower testes weight) ND in maternal weight gain, litter size, fetal weight and fetal mortality
Spence[51]	SD rats	22	L-753,037	5/20/40	13-lactation	21-24	ND in maternal weight gain, litter size, fetal weight, CA and fetal mortality
				PE/FGR models			
Morris[32]	SD rats	x	ABT-627	5	12-19	x	ND in fetal- and placental weight and fetal mortality
Thaete[33]	SD rats	12	FR-139317	6	17-21	21	Treatment normalized hypoxia-induced maternal-, fetal- and placental weight reduction ND in litter size and fetal mortality
Thaete[34]	SD rats	6	A-127722	0.01/0.1/1/5/10	14-21	21	Treatment partly attenuated L-NAME induced FGR ND in litter size and fetal mortality
		6	FR-139317	6/12/18	14-21	21	Treatment partly attenuated L-NAME induced FGR ND in litter size and fetal mortality
		6	A-182086	0.3/10	14-21	21	↓ fetal- and placental weight at 10 mg ND in litter size and fetal mortality
				Knockout models			
Clouthier[42]	ET_AR knockout mice	x	NA	NA	NA	18.5	↑ CA (100% CF and CD) 100% fetal mortality

Table S1. Overview of animal studies reporting administration of ERAs during pregnancy (continued).

Study	Population	N	ERA	Dosage (mg/kg/day)	Treatment period (GD)	Sacrifice / delivery (GD)	Outcome*
Kurihara[43]	ET-1 knockout mice	x	BQ-123	0.96 mg/day	7 days‡	x	↑ CA (CD)
					Other		
Ruest[49]	129S6 mice	x	TBC-3214	100	8	18.5	↑ CA (10% minor CF)
					8-8.5		↑ CA (83% CF)
					8.5		↑ CA (48% minor CF)
					8.5-9		↑ CA (100% CF)
					9		↑ CA (100% CF)
					9-9.5		↑ CA (97% CF)
					9.5		↑ CA (9% minor CF)
					9.5-10		↑ CA (37% minor CF)
					10		↑ CA (4% minor CF)
Thaete[82]	SD rats	x	ABT-546	20	14-21	21	ND in litter size, fetal weight and fetal mortality
		x	FR-139317	12	12-21	21	ND in litter size, fetal weight and fetal mortality

*treated group vs. control group; †all pups died due to patent ductus arteriosus; ‡started at gestational day 5-8; ↑ = significantly increased; ↓ = significantly decreased; x = not described. Abbreviations: bd = twice daily; CA = congenital abnormalities; CD = cardiac; CF = craniofacial; ERA = endothelin receptor antagonist; ET_AR = endothelin type A receptor; ET-1 = endothelin-1; FGR = fetal growth restriction; GD = gestational day; NA = not applicable; ND = no difference; NZ = New Zealand; SD = Sprague Dawley