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Erlandsson, Lena; Nääv, Åsa; Hennessy, Annemarie; Vaiman, Daniel; Gram, Magnus; Åkerström, Bo; Hansson, Stefan

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Inventory of novel animal models addressing etiology of preeclampsia in the development of new therapeutic/intervention opportunities

Lena Erlandsson[1], Åsa Nääv[1], Annemarie Hennessy[2], Daniel Vaiman[3], Magnus Gram[4], Bo Åkerström[4] and Stefan R. Hansson[1]*

[1]Obstetrics and Gynecology, Institution of Clinical Sciences, Lund University, Lund, Sweden.

[2]School of Medicine, Western Sydney University, Campbelltown, New South Wales, Australia

[3]INSERM U1016, CNRS UMR8104, Faculté de Médecine, Institut Cochin, Paris, France.

[4]Infection Medicine, Institution of Clinical Sciences, Lund University, Lund, Sweden

*Correspondence: Dr Stefan Hansson, Department of Obstetrics and Gynecology, Institute for Clinical Sciences, Lund University, BMC C14, Klinikgatan 28, SE-221 85 Lund, Sweden. Stefan.Hansson@med.lu.se

Running head: Models of preeclampsia etiology and intervention

Abstract

Preeclampsia is a pregnancy-related disease afflicting 3-7 % of pregnancies worldwide and leads to maternal and infant morbidity and mortality. The disease is of placental origin and is commonly described as a disease of two stages. A variety of preeclampsia animal models have been proposed, but all of them have limitations in fully recapitulating the human disease. Based on the research question at hand, different or multiple models might be suitable. Multiple animal models in combination with in vitro or ex vivo studies on human placenta together offer a synergistic platform to further our understanding of the etiology of preeclampsia and potential therapeutic interventions. The described animal models of preeclampsia divide into four categories 1) spontaneous, 2) surgically induced, 3) pharmacologically/substance induced, and 4) transgenic. This review aims at providing an inventory of novel models addressing etiology of the disease and or therapeutic/intervention opportunities.

Keywords: α1-microglobulin, hypertension, placenta, proteinuria, treatment opportunities, two-stage model

1. Introduction

Preeclampsia is a pregnancy-related disease afflicting 3-7 % of pregnancies worldwide and leads to maternal and infant morbidity and mortality. Preeclampsia is described to have a placental origin that results in systemic effects in the mother. A variety of preeclampsia animal models have been proposed, but all of them have limitations in fully recapitulating the human disease[1]. The human placenta is unique among species and its function has been suggested to play a central role in the development of preeclampsia. Removal of the placenta is believed to be crucial for the resolution of the symptoms[2], and has led to the theory of a placenta-derived factor as a culprit. The disease evolves in two stages[3]. Stage one occurs during the formation of the placenta with a defective and shallow invasion of the trophoblasts into the uterine muscle layers failing to remodel the spiral arteries[4]. This contributes to a reduced utero-placental blood flow, which can result in fetal intra-uterine growth restriction (IUGR), seen in one of four preeclampsia cases. Inadequate blood flow gives rise to a reduced oxygen delivery and oxidative stress, which further aggravates placental vascular dysfunction[5]. Stage two consists of the clinical manifestations, i.e. hypertension and proteinuria, appearing from 20 weeks of gestation onwards. Early onset preeclampsia is in general more severe than late onset, and is associated with more placenta pathology than late onset preeclampsia. As the disease progresses, angiospasm and brain edema may cause severe epileptic seizures –eclampsia[6]. The renal disturbances seen in preeclampsia lead to reduced glomerular filtration rate and proteinuria. Glomerular endotheliosis is pathognomonic for preeclampsia[7]. General endothelial dysfunction, reduced vasodilatation and increased peripheral resistance are also vascular hallmarks of preeclampsia[8].

The placenta is an organ with extremely high evolutionary diversity among animal species. Hence, an animal model that fully reflects the human placenta does not exist[9, 10]. The majority of

described animal models, however, have placentas that are discoid hemochorial just like the human placenta[9]. Despite this, differences can be found in terms of anatomy, cell types and molecular composition. Few animal models aim to mimic stage one of the disease. The great majority of models instead focus on the second stage, the systemic response and symptoms present in the mother. The ideal animal model should reflect both stages of the disease.

The animal models can be divided into the following four mechanistic categories 1) spontaneous animal models of preeclampsia, 2) surgically induced animal models of preeclampsia, 3) pharmacologically/substance induced animal models of preeclampsia, and 4) transgenic animal models of preeclampsia. Over the past decade a multitude of animal models of preeclampsia have been established and they are already well described in the literature[1, 11-13]. This review aims at providing an inventory of novel models addressing etiology of the disease and or therapeutic/intervention opportunities (Table 1).

2. Animal models of preeclampsia

2.1. Spontaneous animal models of preeclampsia

There are inbred strains of mice and rats that present with spontaneous preeclampsia of various degrees. The BPH/5 mouse, an inbred strain with mildly elevated blood pressure, displays pregnancy-induced characteristics in late gestation resembling those of preeclampsia in humans, including endothelial dysfunction, glomerular lesions, proteinuria and hypertension, as well as feto-placental defects and fetal demise[14]. Defective trophoblast invasion, defects in maternal decidual arteries and an increase in oxidative stress in the placentas preceded the onset of the maternal symptoms[15, 16]. Thus, the model describes events that are linked to both stage one and

stage two of preeclampsia. Treatment with the antioxidant Tempol throughout the pregnancy improved fetal outcome and ameliorated maternal hypertension and proteinuri[16]. It was shown that excessive complement activation in the pregnant BPH/5 females led to increased neutrophil infiltration in the placenta followed by abnormal placental and fetal development as well as reduced vascular endothelial growth factor (VEGF) plasma levels. Inhibition of the complement activation or adenoviral delivery of VEGF early in pregnancy prevented hypertension and proteinuria, and reduced the incidence of fetal resorption[17, 18]. The Dahl salt-sensitive rat strain is a genetic model of kidney disease and hypertension. Females exhibit pregnancy-specific exacerbation of hypertension, proteinuria, placental hypoxia, increased levels of angiogenetic factors and reduced pup and litter size[19]. For both these models, the major criticism is the preexisting hypertension in non-pregnant mice. However, they could be considered as models for superimposed preeclampsia where a preexisting hypertension dramatically increases the risk of developing preeclampsia during pregnancy[20].

2.2 Surgically induced models of preeclampsia

A mechanical model, where the surgical occlusion of the uterine artery or the abdominal aorta results in reduced uterine perfusion pressure (RUPP), has been extensively used to elucidate events occurring during stage two of preeclampsia. The RUPP model has been performed in rats[21], non-human primates[22, 23], sheep[24], rabbits[25], Guinea pigs[26] and dogs[27]. The RUPP rat model has been widely used since it displays a number of typical features of stage two of human preeclampsia such as hypertension, proteinuria and increased plasma and placental levels of angiogenetic markers[21]. This model has recently been used to test therapeutic interventions to alleviate the maternal symptoms. Treatment with sodium tanshinone IIA sulfonate (STS) led to

decreased oxidative stress, but did not improve fetal outcome or maternal blood pressure[28]. Treatment in late gestation with 17-α-hydroxyprogesterone caproate (17-HPC) resulted in decreased blood pressure, decreased levels of circulating $CD4^+$ T cells, reduced uterine artery resistance index and improved litter size[29]. Employing the RUPP model in baboons led to a rapid increase in blood pressure and proteinuria to levels seen in human preeclampsia[23]. There was a rapid rise in soluble fms-like tyrosine kinase 1 (sFlt-1) of a magnitude seen in human preeclampsia; predating the development of proteinuria but timing with the hypertensive response. The response was sustained until delivery. Given the possibility of studying pregnancy over a 4-6 week period, there is an opportunity to study the effect of reducing sFlt-1 while at the same time allowing sufficient time for the syndrome to be controlled without the inevitable delivery of the neonate.

2.3. Pharmacologically/substance induced models of preeclampsia

Several inducible models of preeclampsia are described and the majorities focus on the maternal systemic symptoms in stage two of the disease.

Nitric oxide (NO), a vasodilator, is synthesized by nitric oxide synthase (NOS) from the amino acid L-arginine, and is a vasodilator. Inhibition of NOS in mice or rats by injections of nitro-L-arginine methyl ester (L-NAME) at different gestational stages led to preeclampsia-like symptoms such as hypertension, proteinuria, reduced glomerular filtration rate and IUGR[30, 31]. Concerns have been raised regarding the validity of this model due to uncertainty of the true role of NOS in preeclampsia. However, studies in women with severe preeclampsia have shown a polymorphism in the NOS gene, with certain mutations associated with this group[32]. Both the L-

NAME rat and mouse preeclampsia models have been used for testing therapeutic avenues during pregnancy. Sildenafil treatment was shown to reduce hypertension, proteinuria and fetal demise in both early- and late-onset preeclampsia[33-36] as well as lowering the sFlt-1 and soluble Endoglin (sEng) plasma levels[37]. Other reports have failed to document the positive effects, both in rat and pregnant women suffering from preeclampsia[38, 39]. Although in both cases the treatment was given later in gestation. Resveratrol treatment in L-NAME pregnant rats reduced the hypertension and oxidative stress in placental tissue[40].

Arginine vasopressin (AVP) is highly elevated throughout human preeclampsia pregnancies and as early as the 6th week of gestation it has been proposed as a predictor of preeclampsia[41]. AVP is a peptide hormone that regulates the body's water retention and constricts blood vessels. Thus, at high concentrations it increases the blood pressure, and it has been shown to be elevated in other hypertension disorders. AVP-infusion in mice during pregnancy resulted in both classical maternal and fetal preeclampsia symptoms such as pregnancy-specific hypertension, glomerular endotheliosis, proteinuria and IUGR[41].

Abnormal fatty acid oxidation has been implicated in the pathogenesis of preeclampsia in humans[42]. To investigate this, pregnant mice were injected with beta 2-glycoprotein I (β2GPI) prior to mating and developed preeclampsia-like symptoms such as hypertension, proteinuria and poor pregnancy outcome[43].

Activin A is an anti-angiogenic factor produced by the placenta, and is strongly elevated in plasma from women with preeclampsia. Activin A has therefore been implicated in the pathophysiology of the disease[44]. When activin A was administered at mid-gestation to pregnant

mice it resulted in preeclampsia-like symptoms such as hypertension, endothelial oxidative stress proteinuria and IUGR, and the hypertension and proteinuria were significantly reduced by inhibiting activin A signaling by a low molecular weight activin-receptor-like kinase inhibitor[44].

2.3.1. Fetal hemoglobin-induced model of preeclampsia in pregnant rabbit

The preeclampsia placenta has an increased production and accumulation of cell-free fetal hemoglobin (HbF)[45], resulting in damage to the placenta barrier and consequent leakage of cell-free HbF into the maternal blood circulation[46]. Extracellular hemoglobin (Hb) and its metabolites induce oxidative stress, which may lead to acute renal failure and vascular dysfunction seen in preeclampsia[47]. Cell-free HbF could be detected in the maternal circulation as early as 14 weeks of gestation in women that later developed preeclampsia[48]. Thereby, HbF may link the two stages of preeclampsia through damage to the placenta and eventually to the maternal endothelium[49, 50]. A rabbit model of HbF-induced preeclampsia-like symptoms was recently described[51]. By administering species-specific cell-free HbF, the model mimics the human symptoms at stage two of preeclampsia. The dams displayed disrupted placental morphology, proteinuria and renal glomerular lesions. Further examination of the placenta revealed dramatic reduction of the collagen fibers in the extracellular matrix as well as mitochondrial swelling and high levels of apoptotic bodies. The model failed to evoke any increase in blood pressure. In this model, the therapeutic effect of α_1-microglobulin (A1M) was tested. The human plasma and tissue protein A1M has emerged as a potential therapeutic drug candidate in treatment or prophylaxis of diseases or conditions that are associated with oxidative stress[52, 53]. A1M is synthesized in the liver[54] and secreted to the blood[55]. Of high functional importance is that A1M is rapidly equilibrated between the intra- and extravascular compartments[56, 57]. A1M has mechanistic

properties, which contribute to its role as a tissue housekeeping protein and a potential drug candidate. These properties can be summarized as 1) heme-binding, 2) reductase- and 3) radical-trapping[52]. A1M has been shown to protect cells and tissues against internal and external chemical insult[46, 51, 58-61] and postulated to function as a "radical sink". This refers to its ability to continuously clean tissues from free radicals and oxidants, including free heme and radicals generated by extracellular Hb, heme and iron, by binding, neutralizing and transporting them to the kidneys for degradation[52]. A1M treatment of the preeclampsia rabbits ameliorated the proteinuria and reversed the increased glomerular sieving coefficient in kidney. The A1M-treated animals also displayed a significant reduction of the structural and cellular damages seen in placenta and kidney (Figure 1).

2.3.2. Starvation-induced model of preeclampsia in pregnant ewes

In a pregnant ewe animal model, starvation induces preeclampsia-like symptoms by causing hemolysis with subsequent release of cell-free Hb[62]. In a tailored version of the model, with a reduced time of starvation[61], the exposure to Hb and its metabolites resulted in tissue damage in placenta, with an almost complete elimination of the collagen fibers as well as cellular damages. Structural damages were also observed in kidneys combined with an increase in glomerular sieving coefficient indicating a defect filtration barrier. However, the ewes did not manifest any significant elevation of blood pressure. Intravenous infusion with A1M ameliorated the structural tissue damages seen in both kidney and placenta, as well as restored the glomerular filtration rate in the kidney[61].

2.3.3. *Induced models of preeclampsia in baboons*

The use of non-human primates for study of human pregnancy is predicated on several basic principles related to physiological comparisons[63]. These are singleton pregnancy, upright posture, antigravity blood flow via two uterine arteries arising from the iliac circulation with no co-lateral (or ovarian) blood flow, and single disc placentas[13, 63]. Most importantly though, the formation of placental cell structures that relate to placental metabolic transfer and oxygenation, and likely cell signaling, are common to armadillos, guinea pigs, baboons and humans. This formation is hemomonochorial placentation, which has the lowest number of cell layers between fetal blood flow and the maternal blood-derived supply, i.e. fetal endothelium and trophoblast layer[9, 12]. Baboons have featured in studies of NO, Interleukin-10 (IL-10), TNF-α, and more recently sFlt-1 as an angiogenic pathway inhibitor in pregnancy. In pregnant baboons, treated with a NO inhibitor, the effect on blood pressure was not of the magnitude seen in other species[64, 65]; however, studies of cytokine imbalance have shown that anti-IL-10 caused a low grade but highly significant increase in blood pressure[66], mimicking reduced IL-10 levels seen in preeclampsia[67]. The effect of low dose TNF-α infusion was proteinuria and hypertension[68]. These effects mimicked those seen in rodent studies, linking an inflammatory response to preeclampsia[69]. This was consistent with the studies of human disease, in which patients have been shown to have heightened cytokine production profiles in serum[70] and in placental tissue[71]. Therefore, the capacity to utilize this model to dissect further pathway interactions has increased comparative validity[72].

2.4. Genetically modified models of preeclampsia

Several mouse knockout models display preeclampsia-like characteristics and capture events during stage one and/or stage two. Indoleamine 2,3-dioxygenase (IDO) regulates endothelial-

derived relaxing factors and T-cell activity and the IDO knockout mouse show symptoms of preeclampsia such as proteinuria, mild hypertension, IUGR and glomerular endotheliosis[73]. Interleukin-4 (IL-4) is an anti-inflammatory cytokine and the IL-4 deficient mice display mild preeclampsia-like symptoms during pregnancy including mild hypertension, proteinuria, increased levels of pro-inflammatory cytokines and placental inflammation[74]. IL-10 has been shown to support trophoblast-driven endovascular crosstalk, and pregnant IL-10 knockout mice exposed to hypoxia demonstrate a full spectrum of preeclampsia-like symptoms such as placental injury, renal pathology, proteinuria and hypertension[75]. High temperature requirement A1 (HtrA1) protein is expressed by trophoblast precursors in the placenta and abnormal levels have been observed in women with preeclampsia[76]. Pregnant HtrA1 knockout mice have reduced placental size, pathological changes to the spiral arteries and IUGR[77]. This model also displays impaired remodeling of the maternal arteries, which might suggest that HtrA1 plays a role in stage one of preeclampsia.

In addition to knockout models, various transgenic models in both rats and mice have been established. In a transgenic rat model, female rats transgenic for the human angiotensinogen gene are crossed with male transgenic for the human renin gene, and the pregnant females exhibit typical preeclampsia symptoms such as hypertension, IUGR and proteinuria[78]. This is not the case in the reverse mating. In this model, an increase in regulatory T cells by induction resulted in improved fetal outcome but had no effect on maternal proteinuria or hypertension[79].

A variety of genetic modification is the introduction into rats or mice of adenoviral or lentiviral vectors expressing various proteins. The protein sFlt-1 is an antagonist for VEGF and increased in preeclampsia[80]. Overexpression of sFlt-1 via the administration of viral vectors results in

pregnancy-specific proteinuria and hypertension in mice and rats[81, 82], which was alleviated by the co-administration of VEGF[81]. Similar to this, it was recently shown that removal of excess s-Flt-1 from women with preeclampsia, by plasma apheresis, ameliorated the symptoms and prolonged the pregnancy[83, 84]. Mice subject to viral overexpression of sFlt1 were challenged two months post-partum with an uni-lateral carotid injury, resulting in enhanced vascular remodeling and vessel fibrosis in the preeclampsia-exposed mice[85]. This model could contribute to research regarding the elevated risk of cardiovascular disease seen in women who have had preeclampsia.

2.4.1. STOX1 transgene mouse model

A study of family cases of preeclampsia identified more than 20 genome regions with mutations involved in the disease, one of these has been identified in the Storkhead box 1 (STOX1) gene[86]. Overexpression of STOX1 altered gene expression in a trophoblast cell line, strongly correlating with the transcriptional alterations seen in the preeclamptic placenta[87]. In the STOX1 transgenic mouse model, pregnant female mice recapitulate the human preeclamptic phenotype with hypertension, proteinuria, and increased plasma levels of the anti-angiogenic proteins sFlt-1 and sEng[88]. Moreover, the mice present with alterations of the kidney structure, reminiscent of the renal endotheliosis seen in preeclampsia. Hence, this model re-capsulate both of the stages in preeclampsia, stage one by trophoblast interference in the feto-placental unit, and stage two through its systemic effects on the mother. Since it has recently been shown that STOX1 modulates mitochondrial function, hypoxia response, and the expression of genes involved in oxidative stress, the effects of STOX1 is probably associated with an increase of the oxidative stress[89]. More precisely, STOX1 appears to modulate the balance between oxidative and nitrosative stress. Furthermore, recent results have also shown endothelial cell-deregulation of 2000 genes that are linked to oxidative stress, cardiac hypertrophy and down-regulation of the

cell cycle[90]. In summary, by covering both stages of the disease, the STOX1 transgenic mice constitute a strong model for investigating the etiology of preeclampsia, as well as testing original therapeutic avenues.

3. Summary

Preeclampsia models that involve impaired trophoblast invasion and placentation are a recent contribution to the scientific literature. In the majority of cases, models inducing preeclampsia through experimental interventions fail to capture processes leading to the abnormal placentation postulated to be the core element of the pathophysiology of preeclampsia. Targeting stage two of the disease, namely the maternal symptoms, offers opportunities to evaluate therapeutic options to alleviate the maternal symptoms. However, it sheds little light on the actual etiology of preeclampsia. Based on the research question at hand, different or multiple models might be suitable. Multiple animal models in combination with *in vitro* or *ex vivo* studies on human placenta together offer a synergistic platform to further our understanding of the etiology of preeclampsia and potential therapeutic interventions.

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Table 1. Four mechanistic categories of animal models of preeclampsia addressing etiology of the disease and or therapeutic/intervention opportunities

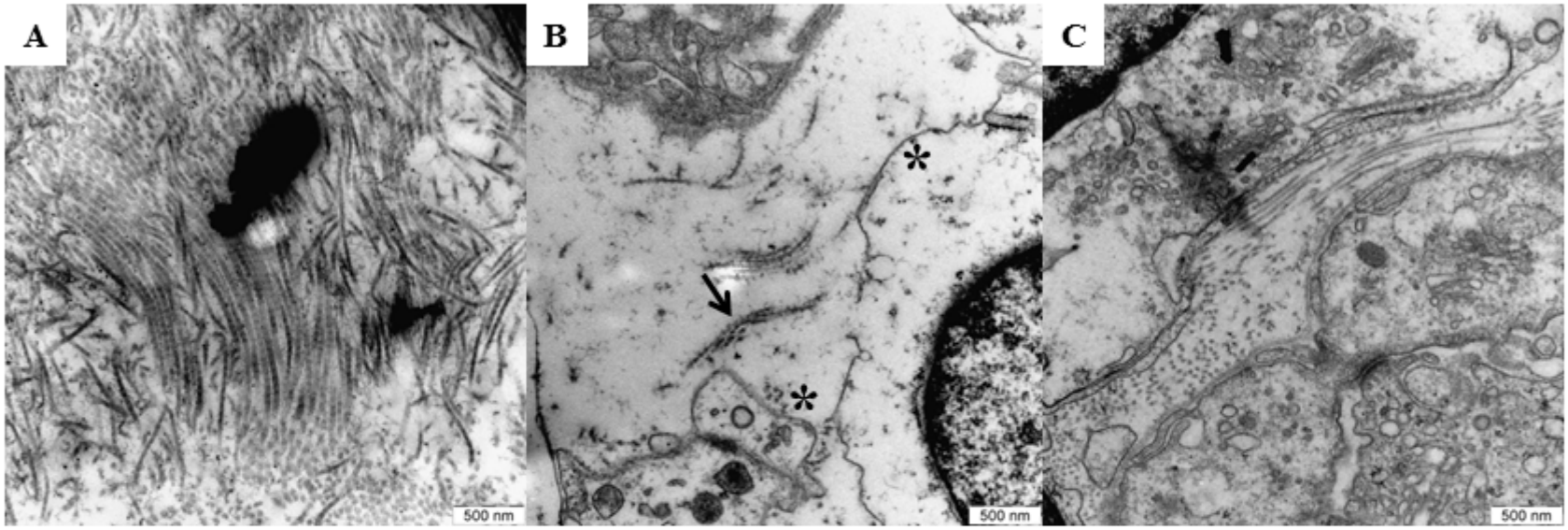
Mechanism	Species	Stage 1	Stage 2	Therapeutic intervention
Spontaneous	Mouse	BPH/5		Tempol
	Mouse	BPH/5		Inhibition of complement
	Mouse	BPH/5		VEGF
	Rat	Dahl S		
Surgical	Rat		RUPP	STS
	Rat		RUPP	17-HPC
	Baboon		RUPP	
Pharmacological	Mouse		L-NAME	Sildenafil
	Rat		L-NAME	Sildenafil
	Rat		L-NAME	Resveratrol
	Mouse		AVP	
	Mouse		β2GPI	
	Mouse		Activin A	Inhibitor
	Rabbit		HbF	A1M
	Sheep		Starvation	A1M
	Baboon		NO inhibitor	
	Baboon		Anti-IL-10	
	Baboon		TNFα	
Genetic	Mouse		IDO (ko)	
	Mouse		IL-4 (ko)	
	Mouse	IL-10 (ko)		
	Mouse	HtrA1(ko)		
	Rat		Angio/Renin(tg)	Induction of T-reg
	Mouse	STOX1(tg)		
	Mouse		sFlt1 (vector)	VEGF
	Rat		sFlt1 (vector)	

β2GPI, beta-2-glycoprotein I; A1M, α_1-microglobulin; AVP, arginine vasopressin; HtrA1, high temperature requirement A1; HbF, fetal hemoglobin; 17-HPC, 17-α-hydroxyprogesterone caproate; IDO, indoleamine 2,3-dioxygenase; $_L$-NAME, nitro-$_L$-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; RUPP, reduced uterine perfusion pressure; sFlt-1, soluble fms-like tyrosine kinase 1; STOX1, Storkhead box 1; STS, sodium tanshinone IIA sulfonate; VEGF, vascular endothelial growth factor.

Figure legends

Figure 1. A1M treatment ameliorates the structural damages caused by cell-free HbF in rabbit placenta

Transmission electron microscopy of placental tissue from HbF-infused pregnant rabbits. (A) Control rabbits showing normal placental tissue with extracellular matrix filled with dense bundles of collagen fibers. (B) HbF-infusion causes loss of collagen fibers (indicated by arrows) together with severe damage to the extracellular matrix, extracellular apoptotic bodies, cell debris and a lot of empty extracellular space (indicated by stars). (C) The structural damages were significantly normalized by A1M treatment, with normal bundles of collagen fibers, normal electron dense barrier and reduced numbers of apoptotic bodies in the extracellular space. Scale bar 500 nm. Image modified from Naav et al. (2015).[51]


A
500 nm
B
500 nm
C
500 nm