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ABSTRACT

Preeclampsia is a hypertensive disorder of pregnancy and the clinical manifestation of severe endothelial dysfunction associated with maternal and foetal morbidity and mortality. The *primum movens* of the disease is the defect of invasion of the uterine arteries by foetal syncytiotrophoblasts, which causes a maladaptive placental response to chronic hypoxia and the secretion of the soluble form of type 1 vascular growth endothelial factor receptor, also called soluble fms-like tyrosine kinase 1 (sFlt-1), the major player in the pathophysiology of the disease. Among its different effects, sFlt-1 induces abnormal sensitivity of the maternal vessels to the vasoconstrictor angiotensin II. This leads to the hypertensive phenotype, recently shown to be abrogated by the administration of sildenafil citrate, which can potentiate the vasodilatory mediator nitrite oxide. This review focuses on the mechanisms of maternal endothelial dysfunction in preeclampsia and discusses the therapeutic window of sildenafil use in the context of preeclampsia, based on the results from preclinical studies and clinical trials. Safety issues recently reported in neonates have considerably narrowed this window.

Keywords: angiotensin II, nitric oxide, preeclampsia, sildenafil, sFlt-1

INTRODUCTION

Preeclampsia: a worldwide syndrome without a cure

Preeclampsia is a clinical syndrome occurring after 20 weeks of gestation and typically characterized on the maternal side by new-onset hypertension and proteinuria (>300 mg/day) [1] and other maternal organ dysfunctions, such as renal failure, liver microangiopathy, neurological or haematological complications, utero-placental dysfunction or foetal growth restriction. The pathophysiology relies on a defective invasion of uterine arteries by the extravillous cytotrophoblasts (of unknown causes) [2]. A history of preeclampsia is found in up to 12% of cases of intrauterine growth retardation (IUGR) and is associated with preterm birth and foetal death, particularly in its early-onset presentation [3]. The health and economic burden associated worldwide with this syndrome are considerable since 2–8% of pregnant women globally will develop preeclampsia, mostly after 35 weeks of gestational age [3, 4]. Immediate maternal mortality due to preeclampsia is estimated to be as high as 50 000 deaths each year, the majority of which are in low-income countries. Longer term, preeclampsia (especially early-onset preeclampsia and recurring preeclampsia) is also associated with an increased risk of cardiovascular events, including fatal ones [5–9]. Beyond this, preeclampsia also increases the cardiovascular risk profile in the offspring [10], while hypertension, stroke and cognitive impairment are more frequent [11–13]. However, this issue is complex since lifestyle and genes overlap here.

To date, no specific therapeutic intervention has been proven to reduce any of these risks, short or long term and, except for placental delivery, caregivers are left with very few preventive or curative options. Pre- and postpartum hypertension are routinely treated with nonspecific, short-life agents, such as nicardipine, labetalol and alpha-methyldopa.

Endothelial dysfunction in preeclampsia: angiogenic imbalance and angiotensin II sensitivity

Understanding of the pathophysiology of the maternal syndrome has advanced over the last 15 years. In short, it has been repeatedly demonstrated that a preeclamptic placenta secretes several antiangiogenic factors in excess, which contribute to endothelial dysfunction in the mother [4]. It has long been known that the hallmark of such endothelial dysfunction is the exaggerated arterial response to angiotensin II (AngII), whereas pregnancy is assumed to be a state of relative resistance to vasopressors [2].

Among the antiangiogenic factors, soluble fms-like tyrosine kinase 1 (sFlt-1) is central to the maternal phenotype. sFlt-1 is the soluble form of type 1 vascular endothelial growth factor receptor (VEGFR-1) and acts by trapping and neutralizing both vascular endothelial growth factor A (VEGF-A) and placental growth factor (PlGF), two pro-angiogenic growth factors [14]. Its pathogenic role was first demonstrated in a seminal clinical trial in 2003 in a rat model of preeclampsia shortly after the observation by a team of oncologists that bevacizumab, a monoclonal antibody targeting VEGF-A, like sFlt-1, was frequently complicated by a preeclampsia-like syndrome [15, 16]. Strategies that aim to reduce sFlt1 concentration in maternal blood using (nonspecific) extracorporeal devices have shown conflicting results and are not routinely prescribed at the bedside [17, 18].

Sildenafil as a new hope in the search for a cure for preeclampsia

Recently Burke *et al.* [19] demonstrated that in the sFlt-1-induced mouse model of preeclampsia, an increased concentration of sFlt-1 was mechanistically involved in abnormal AngII sensitivity and that this abnormal sensitivity could be abrogated by the administration of sildenafil citrate (SC), a phosphodiesterase 5 (PDE5) inhibitor. By diminishing the PDE5-mediated catabolism of cyclic guanosine monophosphate [cGMP; the biological mediator of the potent vasodilator nitric oxide (NO)], SC artificially increases NO-induced vasodilation (Figure 1). This is particularly important in the setting of preeclampsia, where the bioavailability of NO is reduced [22] and is thought to lead to hypertension, platelet aggregation and cellular inflammation.

SC was first developed to treat heart failure, but it eventually became the drug of choice to treat erectile dysfunction and then pulmonary hypertension [23]. Of note, SC has no effect on female sexual dysfunction [24]. Based on the observation that vessels from preeclamptic women have a preserved vasodilatory response to NO, PDE5 inhibitors were actually tested in the setting of preeclampsia as early as 2004 [25]. Since then, promising *in vitro* and *in vivo* animal studies have been published, but in the clinical trials testing SC, the outcome of women with preeclampsia was less favourable. In addition, major safety issues regarding neonates have recently been published and were widely reported in the lay press. This review aims to clarify the perspectives afforded by the most recent experimental and clinical studies. We performed a literature search using the PubMed database, with PDE5, sildenafil, preeclampsia, foetal growth retardation and pregnancy as Medical Subject Headings (MeSH) keywords and with no date limitation.

PREECLAMPSIA: FROM PLACENTA DYSFUNCTION TO MATERNAL PATHOLOGICAL ENDOTHELIUM

In endothelial cells, NO is a gas produced from L-arginine by endothelial NO synthase (eNOS), and its biological effect on smooth muscle cells is mediated by cGMP (Figure 1). cGMP decreases calcium content that induces relaxation of the vasculature [23]. AngII acts in just the opposite way: the binding of its receptor (ATR1) induces phospholipase C and D (PLC, PLD), which increases intracellular calcium content through lipid messengers, and induces a potent contraction of smooth muscle cells. In preeclampsia, endothelin-1 production by the endothelium in response to AngII fuels the same signalling pathways of AngII in smooth muscle cells through the endothelin receptor type A (ET_A), thus potentializing its vasoconstrictor effect (Figure 2).

AngII is produced from the cleavage of angiotensinogen into angiotensin I (AngI) by renin and then converted into AngII by angiotensin-converting enzyme (ACE). When transgenic mice expressing human angiotensinogen are fertilized with transgenic males expressing human renin (with the consequent placenta also expressing human renin), a preeclamptic phenotype is seen [28]. Although the clinical significance is poorly understood, some preeclamptic women display activating autoantibodies directed against ATR1, which is capable of inducing its heterodimerization with the bradykinin β2-receptor and which results in an increased response to AngII (despite lower levels in preeclamptic patients) [29, 30]. A state of relative vasodilation is another potential mechanism for the loss of resistance to vasopressors, particularly to AngII, observed in preeclampsia compared with normal pregnancy [31]. Interestingly, this abnormal sensitivity to AngII lasts beyond delivery: it is still observed in the skin microvasculature of women with a recent (6 months) history of preeclampsia and is borderline significant for mean arterial pressure 5 years after the event [32, 33]. Nevertheless, targeting AngII or the use of ACE is not realistic in pregnant women because of associated severe cardiac and renal defects in the offspring [34, 35].

Conversely, pregnant women exhibit increased activation of eNOS (through increased phosphorylation); this advantage is lost, however, at the very beginning of preeclampsia [19, 36]. Increased production by preeclamptic placentas of soluble endoglin (sENG), the soluble form of the endothelial receptor of transforming growth factor beta (TGF-β), is involved in reduced eNOS activity [29]. By trapping TGF-β and VEGF, sENG and sFlt-1, respectively, thus both contribute to impaired eNOS phosphorylation.

Rats in which preeclampsia is induced by the viral administration of human sFlt-1, sensitivity to AngII is reestablished [19]. In another model of (mild) preeclampsia, induced by a systemic deficiency in catechol-*O*-methyl transferase (COMT), mice also displayed AngII hypersensitivity [37] and an increase (∼20% at Day 17 of gestation) in sFlt-1 concentration [38]. COMT is the enzyme that catalyzes the conversion of 2-hydroxyestradiol into 2-methoxyestradiol (2-ME). Mechanistically, 2-ME induces proliferating peroxisome-activated receptor gamma, a transcription factor that represses ATR1 [37, 38]. COMT-deficient mice logically exhibit an increased sensitivity to AngII. In both models of preeclampsia—COMT knockout and human sFlt-1 overexpression—SC reduces this vascular hypersensitivity to vasoconstrictors [19, 39].

How exactly NO modulates the endothelial sensitivity to AngII is not fully elucidated. NO decreases the activity of ACE and the conversion from AngI to AngII [40] and also acts as a

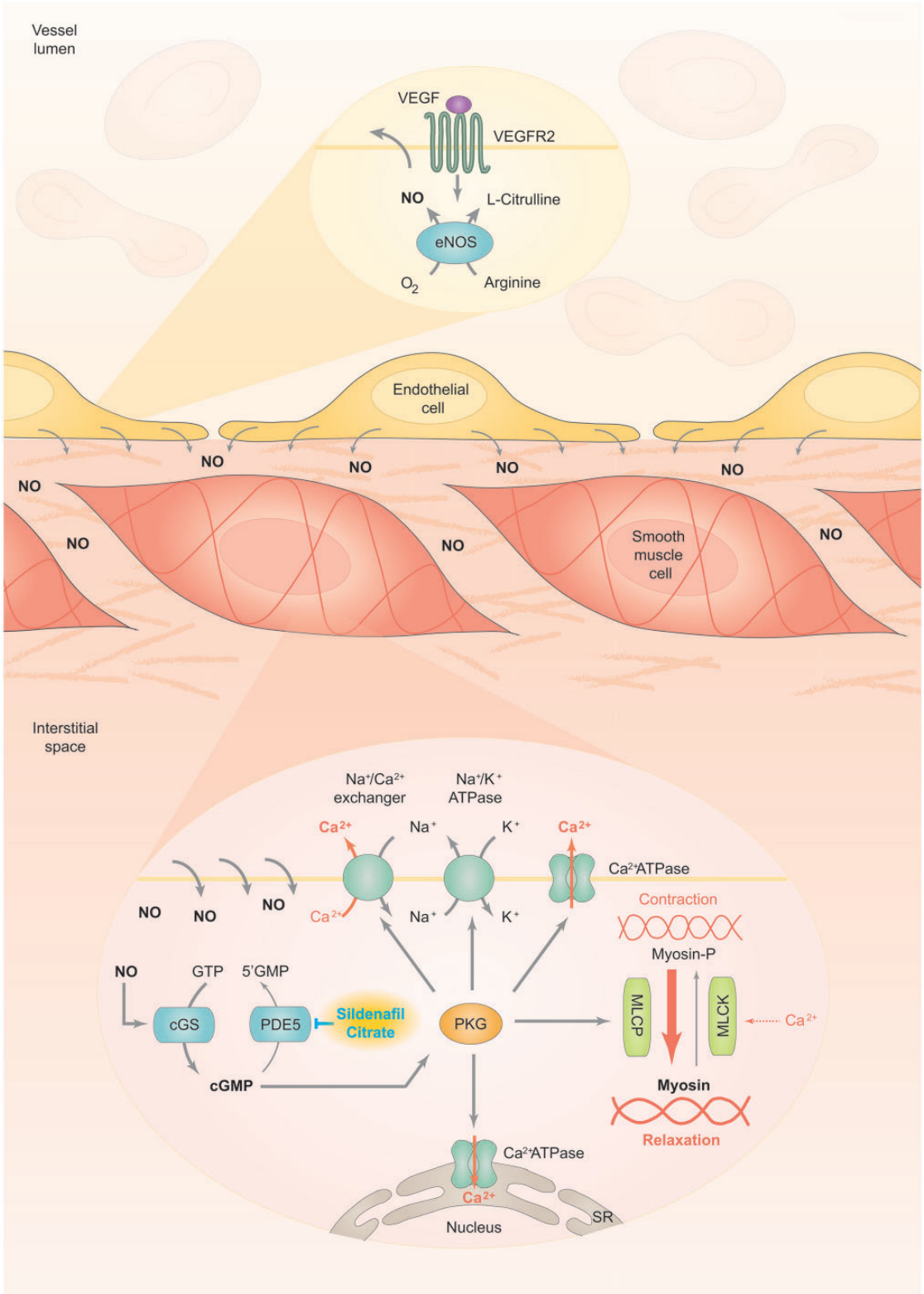

FIGURE 1: Mechanisms of vessel relaxation and effect of SC. Relaxation of the vasculature is dependent on the cross-talk between endothelial and smooth muscle cells. The NO is produced by the eNOS and can be induced by various stimuli, including VEGF through VEGFR-2. The gas freely crosses the membrane of the smooth muscle cell to activate cGMP synthase (cGS). The second messenger then stimulates phosphokinase G (PKG), which leads to the dephosphorylation of myosin through direct activation of myosin light chain phosphatase (MLCP) or inhibition of myosin light chain kinase (MLCK) by a reduction the cytoplasmic concentration of calcium (Ca^{2+}). This last phenomenon relies on PKG-dependent stimulation of the membranous and sarcoplasmic Ca^{2+} adenosine triphosphatase and the Na^{+}–Ca^{2+} exchanger, which pump out the divalent cation from the cytoplasm. By inhibiting PDE5, SC prevents the degradation of cGMP in 5′-GMP, thus sustaining the cellular stock of cGMP and the relaxation state [20, 21]. GTP, guanosine-5′-triphosphate; SR, sarcoplasmic reticulum.

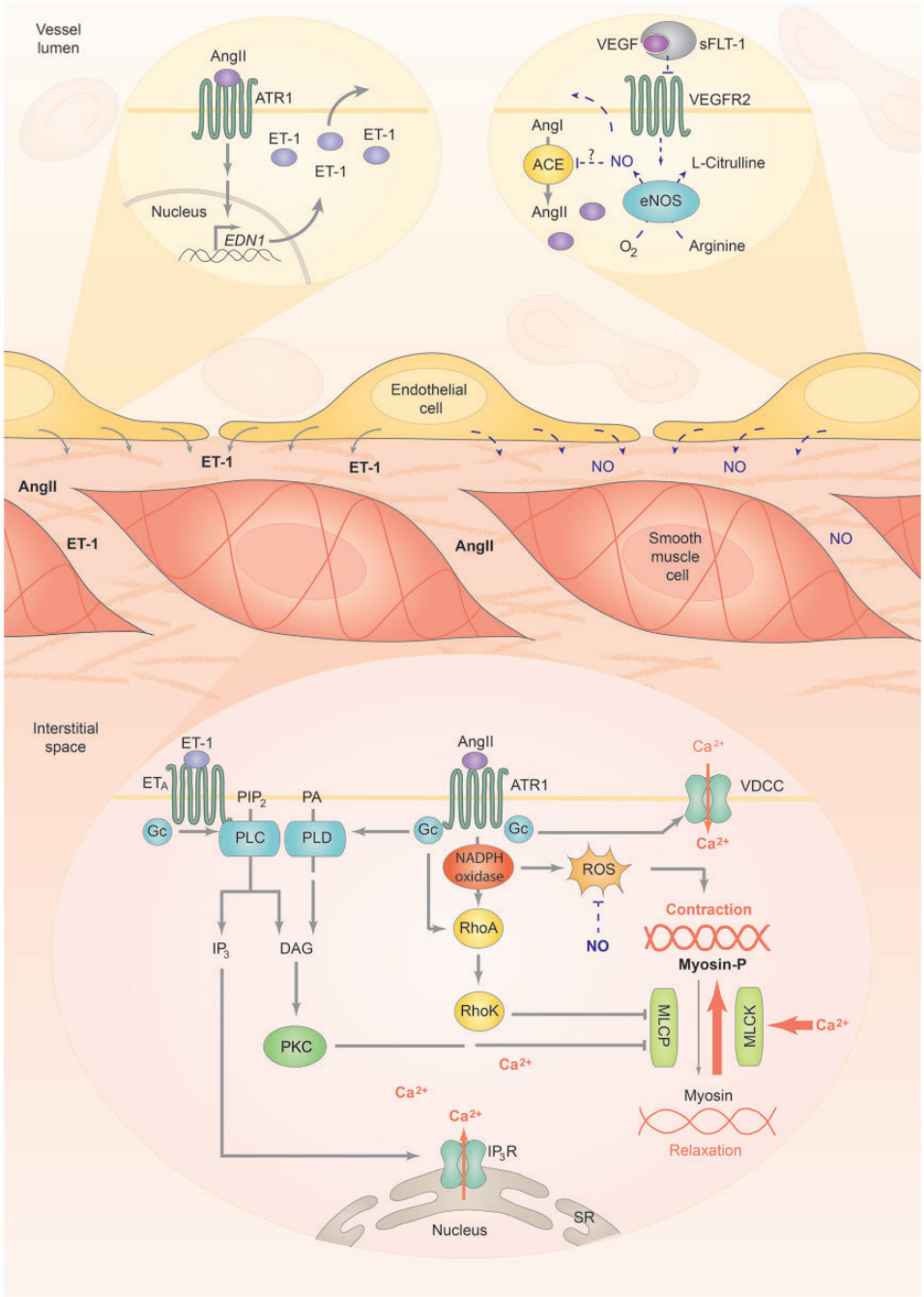

FIGURE 2: Synergy of AngII and endothelin-1 (ET-1) in vascular contraction and their pathologic effect in preeclampsia. The AngII receptor ATR1 is located on both the endothelial and smooth muscle cell membranes. Among its effects, AngII induces the production of ET-1 by the endothelial cells through its receptor ATR1; the two peptidic hormones then participate together in the contraction of smooth muscle cells. The fixation of AngII and ET-1 to ATR1 and ET_A, respectively, leads to stimulation of PLC, PLD via ATR1 or ET_A-coupled protein G. These two enzymes cut membranous phospholipids. The PLC converts phosphatidylinositol 4,5-bisphosphate into inositol triphosphate (IP_3), which opens a specific calcium channel on the sarcoplasmic reticulum (IP_3R), increasing cytoplasmic Ca^{2+} concentration and promoting myosin fibre contraction, thence into diacylglycerol (DAG), which activates phosphokinase C (PKC), which subsequently induces myosin light-chain phosphatase (MLCP) inactivation. The PLD acts in a similar way by indirectly converting the phosphatidic acid into DAG. These two mechanisms are Gq-dependently strengthened by the opening of the voltage-dependent L-type Ca^{2+} channel, with the entrance of Ca^{2+} from the extracellular space into the cytoplasmic compartment, and activation of the Rho signalling pathway, inhibiting MLCP. Altogether these processes contribute to smooth muscle cell contraction [20, 26]. In pregnancy, circulating levels of both AngII and NO are elevated, the combination resulting in a relative resistance to AngII-dependent contraction. However, in preeclampsia, NO contents decrease secondary to the placental secretion of sFlt-1. The soluble receptor traps VEGF, thus preventing endothelial NO production and subsequently leading to more cleavage by the ACE of AngI into AngII, as NO may act as an inhibitor of the enzyme. AngII can also activate Rho member A (RhoA) by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which generates reactive oxygen species (ROS), thus sustaining the state of contraction. The NO deprivation amplifies this last phenomenon as the gas can act as a scavenger for ROS [27].

scavenger of oxidants, which are required for AngII-induced hypertension [41].

RELEVANCE OF SC FOR PREECLAMPSIA: SOME PROMISING PRECLINICAL STUDIES

At present, no specific drug targets sFlt-1, which is why a drug targeting the downstream effects in the preeclamptic maternal endothelium is promising. SC acts through a highly specific inhibition of PDE5, the enzyme that consumes cGMP by converting it into guanosine-5′-triphosphate. cGMP is the first of the second messengers in the NO pathway [23] (Figure 1). The drug was developed in the 1990s as a vasodilatory agent to improve heart failure, but in dose–response Phase I studies it was shown to potentialize the effects of glyceryl trinitrate and to induce penile erection. Eventually erectile dysfunction became the primary indication of SC, with pulmonary arterial hypertension also an indication, but to a much lesser extent (the alveolar endothelium is dependent on NO to become distended in response to inspiration) [23]. Although PDE5 is expressed in almost all tissues, SC selectively acts on only some vascular beds, resulting in a modest reduction in blood pressure in humans, a desirable profile in pregnancy, as a sudden systemic blood pressure drop is deleterious for the foetus [23]. PDE5 inhibitors are thus an interesting alternative to previous strategies that aimed to manipulate the NO pathway in preeclamptic women. NO donors such as glyceryl trinitrate or isosorbide dinitrate or NO precursors such as L-arginine have not proven to be entirely satisfactory [22]. PDE inhibitors were tested *ex vivo* in myometrial biopsies and induced vasodilation in arteries dissected out from preeclamptic subjects, without any effect on placental or omental arteries [25, 42]. In a rat model of preeclampsia induced by suramin (a potent antiangiogenic drug), SC rescued the inhibitory effect on the relaxation induced by nitro-L-arginine methyl ester (L-NAME; a NOS inhibitor) or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (a guanylyl cyclase inhibitor, the enzyme that produces cGMP) on thoracic aorta explants [43]. This efficacy was confirmed *ex vivo* on umbilical arteries isolated from the placenta of preeclamptic pregnancies [44]. In several studies in pregnant rats exposed to L-NAME, the effect of SC on blood pressure control, foetal weight and survival, microalbuminuria (along with a lower quantity of urinary messenger RNA of podocin and nephrin, used here as markers of glomerular injury) has been demonstrated [36–40]. In this model, the pups had impaired learning, also restored by SC, suggesting a protective effect not only on the maternal endothelium but also in the offspring [45]. Furthermore, mice carrying a heterozygous deletion of the *eNOS* gene, affected by a mild hypertensive phenotype, displayed better arterial pressure control and foetal outcome under SC [46]. SC has also been reported to be effective in COMT-deficient mice (a model where preeclampsia is mild but not directly caused by inhibition of NO). It had no impact on blood pressure—and even increased albuminuria—but it improved foetal growth by 20%, most probably by reducing placental arterial resistance [37].

The efficacy of SC might actually go beyond NO metabolism. When given in nonpregnant COMT-deficient mice, SC decreases the serum accumulation of fatty acids and its oxidation intermediates, restoring circulating levels of kynurenine, a precursor of nicotinamide adenine dinucleotide, a rate-limiting compound of oxidative phosphorylation [47]. These results are relevant because mitochondrial dysfunction (on oxidative phosphorylation) is evident in the placenta of preeclamptic women [48]. SC was also found to be of benefit in another model of preeclampsia induced by the clamping of uterine arteries, where the placenta is hypoxic and sFlt-1 is high; of note, the drug had little effect on blood pressure in control rats [49]. Gillis *et al.* [50], however, described the real antihypertensive effect of SC in Dahl salt-sensitive rats, along with decreased proteinuria, decreased uterine artery resistance and a better outcome in pups (increased foetal survival, size and weight).

Timely mechanistic data were provided by the work of Burke *et al.* [19], where SC attenuated sFlt-1-induced preeclampsia and restored a normally low sensitivity to AngII in pregnant mice. By blunting VEGF-driven phosphorylation (hence activity) of eNOS, sFlt-1 increases oxidative stress because NO would normally act as a scavenger of oxidants. In preeclamptic vessels, AngII sensitivity was increased by L-NAME and reversed by SC, confirming the pivotal role played here by NO. Together, these are encouraging data for measurement of the clinical efficacy of SC in humans.

THE USE OF SC AT THE ONSET OF PREECLAMPSIA: CLINICAL STUDIES

To begin with, a meta-analysis pooled the results of preclinical and clinical studies, pointing out some pitfalls in the translation of SC use from rodents to humans: preclinical studies used higher doses (from 4 to 100 mg/kg/day, so at least four times the dose used in clinical trials) and at an earlier stage of pregnancy, when placentation is still ongoing [51]. It should be stressed that prolongation of pregnancy is a major aim since it decreases both morbidity at early and late terms of gestation [52, 53] and cost [54]. However, this is a debatable criterion for outcome since it is dependent on the decision of the investigators to move delivery forward. The ideal (but more complex) criterion would be the preeclampsia-free duration of pregnancy in the case of its introduction before the onset of clinical manifestations. Other PDE5 inhibitors could also be of interest. Vardenafil, for instance, showed an even better vasodilatory effect on umbilical arteries from preeclamptic pregnancies and an increase in the secretion of PlGF by endothelial cells [44, 55]. Tadalafil might be advantageous, as it appears not to cross the placental barrier [56] and was recently found to be safe in a small safety trial (although only eight women were included) [57].

In 2003, years before any *in vitro* or *in vivo* evidence, Downing *et al.* [58] hypothesized that PDE5 inhibitors would be beneficial in preeclampsia. This was based on the idea that accumulation of cGMP would increase vasodilation and NO production would prevent oxidative stress. The first double-blind, placebo-controlled clinical trial of the use of SC in preeclampsia was published 6 years later. Patients were included if they had early-onset preeclampsia (defined here as hypertension plus proteinuria >500 mg/day between 24 and 34 weeks of gestation). The primary outcome was the prolongation of

pregnancy. Gestational age at randomization was 31 + 4 weeks in the SC group and 29 + 0 in the placebo group. The study found no significant difference between groups. Nonetheless, the size of groups (20 subjects each) was probably too small to draw a firm conclusion, with SC, whose half-life is <4 h, given only once a day at a very low dose compared with that used in preclinical studies (20 mg/day, with a progressive increase up to 80 mg if the drug was well tolerated) [59]. Importantly, in this study, where sildenafil use never exceeded 15 days and where mean gestational age at inclusion was ∼30 weeks, there were no safety issues regarding the neonates. In particular, surfactant-deficient lung disease and mortality were comparable between groups. This trial revealed a good SC tolerance profile during preeclampsia and a significant antihypertensive effect: mean diastolic blood pressure decreased from 88 mmHg at randomization to 80 at delivery, while it rose from 90 to 96 in the placebo group [59].

The same group performed a parallel study in which they used pressure myography to analyze the relaxation of the arteries extracted from the umbilical cord and the omentum of the study subjects. They found no difference between groups, showing that the drug had no effect on *ex vivo* samples [60].

Trapani *et al.* [61] recently published similar results in another placebo-controlled trial with a larger cohort of subjects. Inclusion criteria were similar (early-onset preeclampsia), but with a higher dose and a better distribution of SC: 50 mg every 8 h. While in the first study most women were nulliparous, the population of this trial was more heterogeneous, with half being multiparous. Mean gestational age was also ∼30 weeks. A 4-day increase in the duration of pregnancy was observed in the treated group, as well as a significant decrease in mean arterial pressure (from 116.4 ± 5.1 to 100.3 ± 5.6 mmHg at 24 h); a reduced pulsatile index in the uterine and umbilical arteries was also noted (of a modest magnitude compared with the numbers typically observed in healthy pregnancies) without affecting the velocimetric profile of the foetal middle cerebral artery. Outcomes for the baby after delivery were similar to the placebo group, arguing in favour of a selective maternal effect [61, 62]. Respiratory distress syndrome in particular was equivalent between groups, as was death.

Disturbing results from the STRIDER trial

The STRIDER trial (Sildenafil TheRapy in dismal prognosis early onset fetal growth restriction) was meant to present further data on the tolerance and relevance of the prolonged use of sildenafil in early pregnancy (between 22 and 30 weeks of gestation) complicated by IUGR [63]. The UK arm of the trial, conducted in 19 foetal medical units, was published in February 2018 [64]. It failed to demonstrate a beneficial effect of low-dose SC (25 mg three times per day) to prolong pregnancy: time to delivery was 17 days in women exposed to SC versus 18 days in the placebo group. The safety profile of SC was good overall, and perinatal mortality and morbidity were statistically comparable (including the use of surfactant in neonates). However, on 24 July 2018, the lead investigator of the Dutch arm, Dr Wessel Ganzevoort, gave a public interview to *de Volskrant*, a Dutch daily morning newspaper, in which he reported that SC was associated with an increased risk of pulmonary hypertension in newborn babies (17 cases versus 3 in the placebo group) and increased mortality (19 versus 8, of which 11 versus 0 had pulmonary issues). This information led to the suspension of the Canadian arm of the trial [65] and was widely reported in the international nonspecialist press.

CONCLUSION

Preeclampsia is a frequent, potentially fatal syndrome and has long-term consequences for both the mother and her child. Until recently, SC was found to have a satisfactory safety and efficacy profile as an antihypertensive agent in preeclamptic women. However, the Dutch arm of the STRIDER trial, where SC was given early and for up to 4 weeks, rang an alarming warning bell regarding the potential increase in the risk of pulmonary hypertension in neonates, which might be fatal even though a causal link to SC has not yet been proven. Any enthusiasm regarding SC must be tempered by these findings and we must assume a degree of pessimism regarding the future of SC as a drug that improves IUGR and its use before delivery. Whether tadalafil, which does not cross the placental barrier, has similar antihypertensive efficacy and safety in preeclamptic women is uncertain at this time. Very recently, a Phase 2 trial studying the use of tadalafil in IUGR in Japan (UMIN000023778) was stopped prematurely due to the results from the STRIDER trial, but its partial results may give some answers regarding the safety of this other PDE5 inhibitor. However, in our opinion and looking at it from a pathophysiological angle, postpartum use of SC as an antihypertensive drug deserves further investigation.

CONFLICT OF INTEREST STATEMENT

None declared.

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