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Sildenafil citrate decreases sFlt-1 and sEng in pregnant L-NAME treated Sprague–Dawley rats

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ABSTRACT

Objectives: We have previously shown that sildenafil citrate improves various fetal outcomes in pregnant, L-NAME treated, Sprague–Dawley rats. We therefore aimed to identify which component/s of this diverse pathophysiologic cascade is/are improved by this drug.
Study design: This study is a sub-analysis of plasma samples obtained in a previous study in which 24 pregnant Sprague–Dawley dams were divided into three groups ($n = 8$) i.e. the control group (CON), the experimental control group (PRE) where the pre-eclampsia-like symptoms were induced using L-NAME, and the experimental group (SCT) where the pre-eclampsia-like symptoms were once again induced using L-NAME but these animals were treated with sildenafil citrate. On gestation day 20 blood samples were collected in heparin-coated tubes and plasma samples were then analysed for specific variables using commercially available kits for rats.
Results: There was a significant increase in the plasma levels of soluble fms-like tyrosine kinase1 (sFlt-1) in the PRE group (1228.80 ± 116.29 pg/ml) when compared to the CON (774.91 ± 26.81 pg/ml) and SCT (698.98 ± 20.78 pg/ml) groups, respectively ($p < 0.001$). The plasma levels of soluble endoglin (sEng) were significantly decreased in the SCT group (149.47 ± 3.72 ng/ml) when compared to the CON (178.52 ± 5.33 ng/ml) and PRE (183.44 ± 8.294 ng/ml) groups, respectively ($p < 0.01$). Plasma nitric oxide and L-arginine levels showed a decreasing trend in the PRE groups when compared to the control (CON) and treated (SCT) groups, respectively.
Conclusion: Sildenafil citrate reduces the plasma levels of anti-angiogenic factors, sFlt-1 and sEng, in pre-eclamptic (L-NAME induced) Sprague–Dawley rats and may therefore be responsible for the reduction in blood pressure and proteinuria as well as the improved fetal outcomes noted in an earlier study.

1. Introduction

Pre-eclampsia is characterized by an abrupt onset of hypertension and proteinuria after 20 weeks of gestation [1,2]. It affects 3–5% of first pregnancies and is characterised by widespread endothelial dysfunction [3]. The etiology of pre-eclampsia is still not clearly understood, but it is known that the pathogenic process begins much earlier than the presenting symptoms, perhaps at the onset of trophoblast invasion and remodelling of the spiral arteries during the first trimester of pregnancy [2,3]. It is thought to be associated with vascular maladaptation in the placental bed due to this failure of the uterine spiral arteries to undergo complete remodelling into wide bore channels resulting in a marked reduction in blood flow to the placenta [2,4,5]. The reduced placental blood perfusion induces a hypoxic state resulting in the release of a variety of substances including trophoblastic debris and necrotic tissue coupled with an excess secretion of anti-angiogenic factors, viz. soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng). Several groups of researchers have proposed that these excess levels of circulating sFlt-1 may cause the maternal syndrome [6–9].

sFlt-1 has been shown to block the effects of the free or physiological active form of vascular endothelial growth factor (VEGF) by inhibiting interactions with both its receptors (VEGFR-1 and VEGFR-2) [10,11]. Similarly, it also inhibits another member of the VEGF family of growth factors i.e. placental growth factor (PlGF), which is produced by the placenta [12]. This subsequently affects virtually every major organ system by causing endothelial dysfunction and systemic vasospasm [13]. If undiagnosed or

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untreated, pre-eclampsia results in major complications to mother and baby [14]. The only known cure at present is delivery of the baby and placenta [1,14].

Endoglin, a cell-surface co-receptor for transforming growth factor (TGF)-β1 and TGF-β3 isoforms, also plays a key role in angiogenesis [15–17]. Circulating sEng has been shown to be elevated in pre-eclamptic compared to normotensive healthy pregnant women [18]. Administration of sEng on its own does not produce the symptoms of severe pre-eclampsia but co-administration with sFlt-1 showed increased proteinuria, severe hypertension and biochemical evidence of the HELLP syndrome. The authors concluded that these soluble factors act in concert to block the proangiogenic effects of VEGF and TGF-β1 and disrupt endothelial integrity, thereby causing considerable vascular damage [19].

A number of animal models have been used to study the pathogenesis of pre-eclampsia [20–26]. We [27] and others [22,23,28] have successfully shown that inhibition of nitric oxide synthase with L-NAME can also be used as a good animal model to reproduce a pre-eclampsia-like syndrome in which there is hypertension, proteinuria and reduced placental and pup mass. We further showed that the administration of sildenafil citrate (SC) in this model led to a significant reduction in pup fatality, coupled with a decrease in high blood pressure and proteinuria and a corresponding increase in pup and placental mass [27].

In this study, we intend to show the molecular mechanism by which L-NAME produces the pre-eclampsia-like syndrome and how SC can be used to reverse some of the changes.

2. Methods

The animal model was described by us previously [27]. Briefly, 24 pregnant Sprague–Dawley dams were randomly divided into three groups as follows: control group [CON] ($n = 8$), experimental control group [PRE] ($n - 8$) and the SC treated group [SCT] ($n = 8$). The pre-eclampsia-like syndrome was induced in PRE and SCT by adding L-NAME (0.3 g/l) [Sigma–Aldrich, USA] to their drinking water from gestation day 1 until day 19, and CON was given normal water only. Administration of sildenafil citrate [Pfizer, UK-92480-10] (10 mg/kg, b.w.) began on gestation day 7 and continued daily until day 19. The CON and PRE groups were given vehicle only (di-methyl-sulfoxide, 0.3 ml). The animals were maintained under standard laboratory conditions on a 12-h light/dark regime and given access to food and their respective drinking water *ad libitum*. Animals were anaesthetized with halothane (Fluothane™) on gestation day 20 and blood samples were obtained via cardiac puncture in heparin coated tubes. The plasma samples were separated and stored at −70 °C and later analysed for this study.

Plasma nitric oxide (NO) levels were measured following the reduction of nitrate to nitrite by an improved Griess method, using a commercially available kit according to the manufacturer's protocol (BioAssay Systems, USA). L-Arginine levels were measured in the plasma by using a chromogen that forms a coloured complex with urea that is produced by arginase activity. This was achieved by a commercially available kit (BioAssay Systems, USA). Plasma levels of angiogenic factors (VEGF and PlGF) and anti-angiogenic factors (sFlt-1 and sEng) were measured by quantitative sandwich enzyme immunoassay techniques using commercially available kits for rats according to the manufacturers protocol (R&D Systems, USA).

All data were subjected to one-way ANOVA and/or the Tukey-Kramer Multiple Comparison Test using the GraphPad Instat (v.05) statistical software package. Results are presented as mean ± standard error of the means (SEM). A probability value of <0.05, was considered statistically significant.

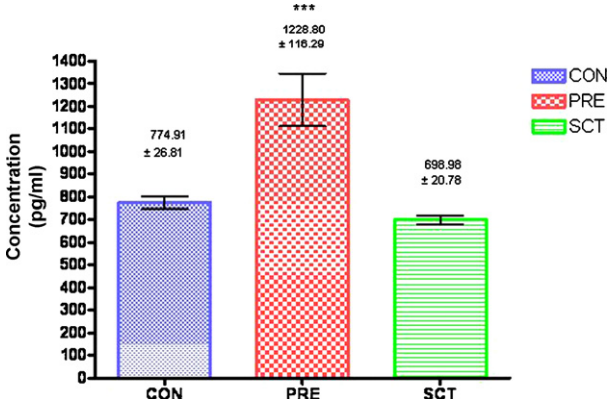

Fig. 1. Plasma sFlt-1 levels for CON, PRE and SCT, respectively. Plasma sFlt-1 levels were determined by quantitative sandwich enzyme immunoassay. The data are expressed in picograms per milliliter (pg/ml) and presented as mean ± SEM, where (***) is $p < 0.001$ for PRE versus CON and SCT.

3. Results

Changes in mean plasma levels of sFlt-1 and sEng are shown in Figs. 1 and 2, respectively. The plasma concentration of sFlt-1 in the PRE group (1228.80 ± 116.29 pg/ml) was significantly elevated when compared to the CON (774.91 ± 26.81 pg/ml) and SCT (698.98 ± 20.78 pg/ml) groups, respectively ($p < 0.001$). There was no statistical significance between the CON and SCT groups. The plasma concentration of sEng in the SCT group (149.47 ± 3.72 ng/ml) was significantly decreased when compared to the CON (178.52 ± 5.33 ng/ml) and PRE (183.44 ± 8.294 ng/ml) groups, respectively ($p < 0.01$). There was no statistical significance between the CON and PRE groups.

The plasma levels of NO in the PRE group (9.87 ± 0.59 μM) were decreased compared to the CON (11.20 ± 1.05 μM) and SCT (11.92 ± 3.70 μM) groups, respectively (Fig. 3). This did not reach statistical significance ($p > 0.05$), however. The plasma levels of L-Arg were decreased in both the PRE (5.08 ± 0.81 μM) and SCT (6.26 ± 0.24 μM) groups compared to the CON group (10.47 ± 2.31 μM) (Fig. 3) but this did not reach statistical significance ($p > 0.05$). There was no significant difference in the plasma concentration of VEGF and PlGF for CON versus PRE and SCT groups, respectively (Fig. 4).

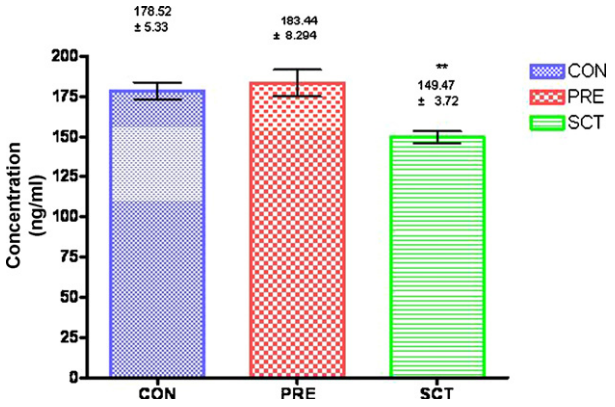

Fig. 2. Plasma sEng levels for CON, PRE and SCT, respectively. Plasma sEng levels were determined by quantitative sandwich enzyme immunoassay. The data are expressed in nanograms per milliliter (ng/ml) and presented as mean ± SEM, where (**) is $p < 0.01$ for SCT versus PRE.