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SEBİLE GÜLER ÇEKİÇ
EDİP GÜVENÇ ÇEKİÇ
DİLEK BAYDAR
MERAL TUNCER
GÜRAY SOYDAN

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Vascular responses of aortic, renal, and uterine arteries in suramin-induced preeclampsia-like syndrome in rats

Sebile Güler ÇEKİÇ¹, Edip Güvenç ÇEKİÇ¹, Dilek Ertöy BAYDAR², Meral TUNCER¹, Güray SOYDAN¹*¹
¹Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey
²Department of Pathology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

1. Introduction
Preeclampsia is a pregnancy-associated disease in which hypertension, proteinuria, placental insufficiency, and fetal growth retardation occurs, and it is one of the leading diseases affecting both maternal and fetal mortality and morbidity (1,2). It is estimated that 7%–10% of all pregnancies are complicated with preeclampsia (3). Despite intense investigations, ethiopathogenesis of the disease is not clear yet (4).

There are several findings of the disease such as abnormal placentation even detected in the early stages of the disease (5,6). Endothelial dysfunction causes increased responses to constrictor agents and reduced relaxations to acetylcholine and other related relaxing agents (7), aggravated inflammatory response (8), disruption in angiogenesis (4,9), and general vasospasm (10). It is shown that trophoblast invasion in the placenta is shallow in preeclampsia (11). From these findings, Redman et al. (3) proposed “two-stage model of preeclampsia”. According to this hypothesis, interruption in trophoblast migration to the spiral arteries and reduced formation of new arteries in the placenta cause reduced placental perfusion. The mediators released during placental hypoxia, generate local inflammation which is called the first (placental) stage. Stage 1 is not sufficient to cause maternal syndrome. Together with the maternal constitution (genetics, accompanying diseases, and behavioral and environmental factors), Stage 1 proceeds to Stage 2 while the defect in placentation causes systemic inflammatory response. The link between these two stages is considered to be oxidative stress and lowered angiogenesis. It is speculated that hypoxia-driven reduction in angiogenic factors such as vascular endothelial growth factor (VEGF) or placental derived growth factor (PIGF) might contribute to some of the maternal abnormalities in preeclampsia (12,13). The deficit in circulating angiogenic factors may initiate a cascade of cellular and molecular events leading to endothelial and vascular dysfunction (14). In accord with this hypothesis, Carlström et al. proposed an animal model in which angiogenesis was inhibited by suramin...
and preeclampsia-like syndrome was developed (15,16). Suramin is a potent angiogenesis inhibitor in rodents (17). It was shown that suramin inhibits VEGF and PI GF which are especially increased during placentation (17). By inhibiting these agents, suramin attenuates placental development in rat pregnancy, approved with the finding that mesometrial area, where maternal and fetal exchange occurs, is decreased in preeclampsia-like syndrome (15). The blockade in the placental development causes reduced placental and renal blood flow, decreased fetal maturity and increased inflammatory response (16).

It has been suggested that release of factors from the placenta in response to ischemia results in endothelial dysfunction of the maternal circulation in humans (7,18). Consistently with human findings, endothelial dysfunction also occurs in aortic rings of rats (19). To our knowledge, functional studies on uterine artery which is important for perfusion of uterus and on renal artery which plays an important role for kidney perfusion have not been conducted before for preeclampsia-like syndrome.

The aim of this study was to produce preeclampsia-like syndrome by suramin administration in rats and to investigate the functional responses in aortic, renal, and also uterine arteries which is important in the nourishment of the placenta and fetus.

2. Materials and methods
All experimental procedures were conducted as per the study protocol approved by Hacettepe University Local Ethics Committee for animal experiments (2009/39). Twenty-four virgin female Wistar albino rats aged 12–20 weeks and weighed 180–230 g were used in the experiments. Animals were sheltered in optimum temperatures (20–23 °C) and 12 h light 12 h dark cycle. Food and water were ad libitum. Rats were caged overnight with males. In the morning, vaginal smears were collected by washing the vagina with a micropipette containing 100 μL of saline, and then 1 or 2 drops were taken on the lamels and evaluated by light microscopy. The day spermatozoa was found in vaginal smear was accepted as the first day of pregnancy. Rat spermatozoa were like commas as seen in Figure 1.

2.1. Groups
There were four groups in the study as control nonpregnant (C), suramin-given nonpregnant (S), control pregnant (CP), and suramin-given pregnant (SP) rats. n values were 5 in C and 6 in other groups. One rat with preterm labor was discarded.

2.2. Blood pressure measurements
Systolic blood pressures were measured daily for 20 days with tail-cuff noninvasive blood pressure monitoring system (Biopac Systems, Goleta, CA, USA). Tails of rats were warmed for 20 min in tail warmer controller (Commat, Turkey). Blood pressures were recorded with data transmission unit MP150 (Biopac Systems). The pressure where the first pulses appear was accepted as the systolic blood pressure. Average of ten consecutive measures was calculated.

2.3. Suramin injection
Rats in the groups S and SP received 100 mg/kg suramin intraperitoneally (i.p.) whereas those in groups C and CP received an equivalent volume of saline on days 10 and 11.

2.4. Measurement of proteinuria and number and weight of fetuses and placentas
On day 20, animals were sacrificed with urethane 1.5 g/kg i.p., and then urine samples were taken for assessment of proteinuria which is determined semiquantitatively with colorimetric urine sticks (Arroy, Japan). According to the color reaction on the urine strips, proteinuria was graded as “0”, “+1” (proteinuria refers to 0–30 mg/dL), “+2” (proteinuria refers to 31–100 mg/dL), and “+3” (proteinuria refers to 101–300 mg/dL). Left kidneys were taken out in 10% formaldehyde for pathological procedures. Then, left renal arteries were removed into physiological salt solution (PSS; composition, mmol/L: NaCl, 118; KCl, 4.6; NaHCO₃, 25; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 1.2; glucose, 10; EDTA, 0.025; pH, 7.4 at 37 °C)-containing petri dishes before uteruses were removed. Uteruses with the surrounding arteries and fat tissue were taken out in a petri dish, counted, and weighed on a sensitive balanced weight. Thoraces were opened and thoracic aortas were isolated in PSS-containing petri dishes and placed in an organ bath.

Figure 1. Vaginal smear of rats. Picture of rat spermatozoa (arrows) in the vaginal smear of female rats on light microscope (100× magnification).
2.5. Preparation of arteries for functional studies

Functional studies were done only in pregnant groups. The thoracic aortas were placed in a 20-mL organ bath and responses were recorded with an isometric force displacement transducer (FT03C, USA) connected to a polygraph (Grass Model 7B, USA) whereas uterine and renal arteries were placed onto 5-mL organ bath and studied by myograph system (610M, Danish Myo Technology, Denmark).

The thoracic aorta was dissected and 5-mm rings were prepared. Two parallel wires were placed in the lumen of the rings and the tissues were mounted into a 20-mL organ bath filled with PSS and aerated with 95% O₂ and 5% CO₂ gas mixture at 37°C. Responses of the aortic rings were measured by a force displacement transducer (FT03C, USA) and recorded by a polygraph (Grass Model 7B, USA). Rings were rested for 30 min under a basal tension of 1 g. After a 30-min equilibration period, the arteries were stimulated with isotonic high-K⁺ PSS (100 mM K⁺) added at the beginning of the experiments to test smooth muscle contractility. The concentration of NaCl was rearranged to obtain an isotonic solution and the final concentrations in the isotonic high-K⁺ PSS (100 mM K⁺) solution were as follows; NaCl: 22.6 mM; KCl: 100 mM; NaHCO₃: 25 mM; MgSO₄: 1.2 mM; KH₂PO₄: 1.2 mM; CaCl₂: 1.25 mM; glucose: 10 mM; and EDTA: 0.025 mM. After the equilibration period contractile responses to cumulative doses of phenylephrine (PE, 10⁻⁹ – 3 × 10⁻⁵ M) were recorded. Tissues were washed, precontracted with submaximal concentration of PE (3 × 10⁻⁷ M) and after the contraction reached a plateau, the relaxations to acetylcholine (ACh, 10⁻¹⁰ – 3 × 10⁻⁵ M) and sodium nitroprusside (SNP, 10⁻¹⁰–3 × 10⁻⁵ M) were obtained.

Uterine and renal rings were isolated and cut into 2 mm long segments. Each segment were threaded onto 2 stainless steel wires (40-μm diameter) and mounted in a wire myograph with four organ chambers containing 5 mL of PSS each in an isometric myograph (610M, Danish Myo Technology, Aarhus, Denmark). The wire myograph bath contained 5 mL of PSS, which was continuously aerated with a gas mixture (95% O₂ and 5% CO₂) in order to maintain the pH at 7.4. After mounting, the vessels were allowed to equilibrate for 30 min at 37°C. Contractile responses, two-way ANOVA for repeated measurements were evaluated with Student’s t-test. For the functional responses, two-way ANOVA for repeated measurements was used to compare concentration–response graphs. All concentration–response graphs were drawn in curve fit via GraphPad Prism 5 program. E_max and pD₅₀ values were calculated using GraphPad Prism 5 and were compared with Student’s t-test. P < 0.05 was accepted as significant.
3. Results

3.1. Physiological changes
The initial weight of rats before pregnancy did not vary among four groups. Maternal weight gain was highest in CP with 83.60 ± 5.00 g (38.32 ± 2.84% of the initial weight) and was lower in SP with 51.40 ± 11.8 (23.81 ± 5.00% of the initial weight; P < 0.05). In nonpregnant rats, suramin treatment did not cause any variations in weight gain (Table 1).

At the beginning of the experiments, all groups had similar systolic blood pressures which were as follows; 97.81 ± 2.73 mmHg in C, 101.15 ± 2.47 mmHg in S, 92.44 ± 1.73 mmHg in CP, and 96.91 ± 2.12 mmHg in SP (Figures 2A and 2B). There were no significant differences in systolic blood pressures in nonpregnant rats on day 20 compared to day 1 (89.51 ± 7.40 mmHg in C and 100.78 ± 3.88 mmHg in S, respectively; Figure 2A). On day 20, systolic blood pressures decreased to 70.15 ± 3.09 mmHg (24.11%) in CP; however, they increased to 134.06 ± 4.80 mmHg (38.33%) in SP, compared to day 1 of the experiment (Figure 2B). Blood pressure differences became significant starting from day 13 (Figure 2B).

The assessment of urine protein levels as median showed an increase from +1 (approximately 10 mg/dL) in CP to +3 (approximately 300 mg/dL) in SP group (Figure 3). In nonpregnants, there was no significant difference in urine protein levels (Figure 3).

There is no difference between the average weights of the kidneys in nonpregnants (0.85 ± 0.05 g in C and 0.92 ± 0.05 g in S). However, a significant increase was found in suramin-given pregnant rats compared to control pregnants (0.79 ± 0.04 g in CP and 0.95 ± 0.04 g in SP). Similar results were obtained when weight of the kidneys are proportioned to the body weight. Thereby the standardized kidney weights were 0.36 ± 0.02 g and 0.45 ± 0.04 g (C and S, respectively), 0.25 ± 0.01 g and 0.37 ± 0.01g (CP and SP, respectively).

The average numbers of fetuses did not differ between groups; however, the average weight of the fetuses and placentas were found to be lower in SP (Table 2).

3.2. Pathological responses
Pathological examination of the kidneys in the suramin-given groups (S and SP) revealed severe tubular injury. Proximal tubules in the renal cortex showed diffuse macrovacular degeneration. Some tubular epithelial cells were lysed with the dissolution of their nuclei. Renal medulla, glomeruli, and blood vessels were unaffected (Figure 4).

3.3. Functional responses
3.3.1. Thoracic aorta
There were no differences between groups in KCl-induced contractions (0.63 ± 0.04 g in CP and 0.57 ± 0.07 g in SP). Concentration-dependent PE contractions were increased in SP (Figure 5A). There were no differences in pD2 values between groups (Table 3). However, E_max for PE was increased in SP compared to CP rats (Table 3).

ACh- and SNP-induced relaxations did not differ in pregnant groups (Figures 5B and 5C). There were no differences in E_max and pD2 values between CP and SP groups in ACh- and SNP-induced relaxations (Table 4).

3.3.2. Renal artery
There were no differences between groups in KCl contractions (3.27 ± 0.45 mN/mm in CP and 2.93 ± 0.47 in SP). Concentration-dependent contractions in PE did not show any difference between groups (Figure 6A); however, 5-HT responses were increased in SP (Figure 6B). There were no differences in pD2 values both for PE and 5-HT in CP and SP rats (Table 3). E_max values for 5-HT was higher in SP (Table 3).

Ach-induced relaxations were decreased in SP (Figure 6C). In the presence of nitric oxide inhibitor, L-NAME (10^-4 M), the relaxations induced by ACh were significantly decreased in CP and SP; however, the difference between groups was abolished in the presence of L-NAME (Figure 6C). pD2 and E_max for ACh were decreased in SP (Table 4). SNP relaxations were same in both groups (Figure 6D), there were no differences between CP and SP in pD2 and E_max values (Table 4).

3.3.3. Uterine artery
There was no difference between groups in KCl contractions (4.17 ± 0.11 mN/mm in CP and 4.34 ± 0.18 mN/mm in SP in CP and SP rats (Table 3). E_max values for 5-HT was higher in SP (Table 3).

Table 1. Weights and weight gain of the rats.

|       | Initial weight (g) | Average weight change (g) | Percentage of weight change (%) |
|-------|-------------------|---------------------------|---------------------------------|
| C     | 218.2 ± 3.5       | +12.2 ± 5.8               | +5.7 ± 2.7                      |
| S     | 214.5 ± 8.8       | +10.2 ± 2.1               | +4.8 ± 1.0                      |
| CP    | 219.2 ± 5.0       | +83.6 ± 5.0*              | +38.3 ± 2.8*                   |
| SP    | 212.2 ± 8.2       | +51.4 ± 11.8*             | +23.8 ± 5.0*                   |

Control nonpregnant (C, n= 5), suramin-given nonpregnant (S, n=6), control pregnant (CP, n=6), and suramin-given pregnant (SP, n=5). *P < 0.05 versus C, # P < 0.05 versus CP.
Figure 2. Daily blood pressure measurements in, A. Control nonpregnant (C), suramin-given nonpregnant (S) and B. Control pregnant (CP), suramin-given pregnant (SP). *P < 0.05 versus Day 1.

4. Discussion
Findings in this investigation demonstrated that exposure to suramin in pregnant rats caused preeclampsia-like syndrome confirmed by physiological, functional, and structural changes. In consistence with previous studies (15,16,19), maternal hypertension, proteinuria, and placental insufficiency occurred in suramin-induced preeclampsia-like syndrome. Increase in systolic blood pressure and proteinuria, decrease in the weight of fetuses and placentas are in support of the model. To our knowledge, this is the first study which investigates functional responses of renal and uterine arteries in this model. Additionally, for the first time, we have shown acute tubular necrosis in the kidneys of suramin-given pregnant rats.

Preeclampsia is a disease that affects both maternal and fetal health due to unknown causes. Redman et al. (3)
suggested a “two-stage model” which proposed a placental defect from the beginning of the pregnancy. This defect, with accompanying factors like placental, maternal, or mental conditions, leads to placental insufficiency (3,8). Suramin-induced preeclampsia-like syndrome seems to fit this hypothesis when compared to other animal models. The models about angiogenesis blockade presented in the last years report that sFlt-1 and sEng injection also cause preeclampsia-like syndrome (21). Nevertheless, the molecules such as sFlt-1 or sEng are the results of an existing problem. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia (22) and symptoms mimicking preeclampsia like hypertension and proteinuria were also observed in nonpregnant rats in this model (21). So the origin of the disease is still unclear. The model used in this study has been thought to be a more appropriate model than those recently described for explanation of the pathophysiology of preeclampsia.

Previously, it was shown that in pregnant rats given 100 mg/kg suramin, blood pressures were elevated, placental and renal blood flow were reduced, and serum ET-1 levels

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**Figure 3.** Proteinuria levels in control nonpregnant (C), suramin-given nonpregnant (S), control pregnant (CP), and suramin given pregnant (SP). *P < 0.05 versus C, †P < 0.05 versus CP.

**Table 2.** Average fetus numbers and weight of fetuses and placentas.

|         | Average number of fetuses per labor | Average weight of placetas (g) | Average weight of fetuses (g) |
|---------|-------------------------------------|--------------------------------|-----------------------------|
| CP      | 11.71 ± 0.94                        | 0.48 ± 0.01                    | 2.63 ± 0.60                 |
| SP      | 11.39 ± 1.11                        | 0.36 ± 0.01*                   | 1.82 ± 0.06*                |

Suramin-given pregnant (SP) compared to control pregnant (CP) rats. *P < 0.05 versus CP.
Figure 4. Microscopic histologic examination of rat kidneys with hematoxilene/eosine stain. A: Nonpregnant control rats. B: Nonpregnant suramin-given rats. C: Pregnant control rats. D: Suramin-given pregnant rats. Normal histologic findings were seen in A and C, whereas acute tubuler necrosis were seen in B and D (arrows). Renal medulla, glomeruli, and blood vessels were unaffected in all groups.

Figure 5. Functional responses of aorta. Contractions induced by phenylephrine (PE; A), and relaxations induced by acetylcholine (ACh; B) and sodium nitroprussiate (SNP; C) in aortic rings in control pregnant (CP) and suramin pregnant (SP) rats. *P < 0.05 versus CP.
were increased. In another study, together with an increase in blood pressure and proteinuria, endothelial dysfunction in aorta were observed in pregnant rats when given 100 mg/kg suramin (19). In both studies, number and weight of the fetuses and placertas were decreased in suramin-given groups. Consistently, in our study, we observed increase in blood pressure and proteinuria. Additionally, decrease in number and weight of fetuses and decrease in weight of placenta, which are signs of placental insufficiency, were observed in suramin-given pregnant rats. Blood pressures measured by tail-cuff method in our study were similar to the finding of telemetric blood pressure measuring system (15). Suramin did not cause an increase in blood pressure in nonpregnant rats.

The ideal system to measure urine protein levels is to use metabolic cages. Since we did not have this system, we measured protein levels with urine sticks from spot urine which may give false-positive or false-negative results with a sensitivity of 32%–46% and specificity of 97%–100% (23). Proteinuria was spotted in control
nonpregnant, suramin-given nonpregnant, and control pregnant rats; however, it was significantly less than that in suramin-given pregnant rats. Suramin caused significant proteinuria in pregnant but not in nonpregnant rats. This finding is concomitant with the finding in the same model measured with metabolic cage. Nash et al. (16) also found proteinuria in nonpregnant and control pregnant rats less than in suramin-given pregnant rats.

Pathological examination of kidneys revealed acute tubular necrosis both in pregnant and nonpregnant rats. It is known that suramin has reversible and dose-dependent nephrotoxic effect (24). However, it is contradicting that suramin caused significant proteinuria in pregnant but not in nonpregnant rats.

The weights of fetuses and placentas were decreased in suramin-given pregnant rats, which is consistent with the previous studies (15,16,19), indicating placental insufficiency. Although we did not examine the placental pathology, Carlström et al. (15) showed that angiogenesis and the mesometrial area were decreased in suramin-given pregnant rats.

Preeclampsia is a kind of disease in which relaxant factors such as NO decrease and vasoconstrictor substances such as ET-1, PE, and TXA₂ increase, which leads to

![Functional responses of renal arteries.](image)
endothelial cell dysfunction (25,26). Unlike previous studies, we could not detect endothelial dysfunction in thoracic aorta. Turgut et al. (19) observed increased responses to PE and decreased vasodilatation in aortic rings. The contradicting finding of thoracic aorta in our study could be due to animal differences.

For the first time in this model, we have observed endothelial dysfunction in uterine and renal arteries. Functional analysis of different arteries showed different properties in this study. Although the responses to KCl, a depolarizing agent, were alike among the groups, some receptor-dependent responses differed in pregnant rats. Responses to many contractile agents (PE, ET-1, and PGF2α) were increased in uterine artery in rats with preeclampsia-like syndrome. Although E_max was increased for PE, ET-1, and PGF2α in suramin-given pregnant rats, no difference was found in pD2 values. Similar pD2 but increased E_max values may indicate an alteration in postreceptor events. These findings were compatible with those of the studies on human isolated myometrial branches of uterine artery in preeclampsia (25,27,28). ET-1 is a potent vasoconstrictor and its level is found to be increased in preeclamptic pregnancies (7,25,26). It was shown that ET-1 levels are increased in line with the severity of the disease and decrease to the normal levels within 48 h after labor (29). In line with the literature, ET-1 responses were increased in our study.

Endothelial dysfunction also occurred in renal arteries in our study. Relaxation by ACh diminished in SP, whereas SNP-induced relaxation did not differ in SP and CP. Furthermore, in the presence of L-NAME, similar ACh relaxations were equal in both groups. These results indicate an endothelial dysfunction through NO mechanism. Since the relaxations did not fully disappear, mediators rather than NO also play a role in the relaxation of the renal artery.

NO is a vasodilatating substance produced by (nitric oxide synthase) NOS from nonessential aminoacid L-arginin. NO is one of the mediators which increases in pregnancy and it was shown that NOS expression and activity are increased in the human uterine artery during pregnancy (30). Moreover, plasma levels of cGMP, secondary messenger of NO, and its metabolites were shown to be increased in pregnancy (31).
preeclampsia, abnormal formation of spiral arteries reduces utero-placental NO production, enhances thromboxane secretion thus causing platelet adhesion. In contrast to physiological changes in pregnancy (32), NOS expression and amount of cGMP were decreased in preeclampsia (33). Attenuation of the relaxation of myometrial arteries taken from preeclamptic women implies endothelial dysfunction (27). In our study, endothelium-dependent responses to ACh diminished in suramin-induced preeclampsia-like syndrome in concord with the human studies. Relaxations to ACh in the uterine arteries diminished in suramin-given pregnant rats, whereas SNP relaxations and ACh relaxations in the presence of L-NAME were similar. These results indicate an endothelial dysfunction through NO mechanism. Since the relaxations did not fully disappear, it may be concluded that mediators rather than NO also play a role in the relaxation of the uterine artery, similar to renal artery in this study.

5. Conclusion
In this study, we showed that preeclampsia-like syndrome occurred with suramin, possibly through inhibiting angiogenesis. Our findings, which showed that endothelial dysfunction occurred in uterine and renal arteries but not in the aorta, are consistent with the human findings of microvascular changes in preeclampsia (34,35). We also showed that this model fits the “two-stage” model in humans and could be used to enlighten the pathophysiology of preeclampsia.

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