

Effect of Toki-shakuyaku-san on the Fetal Development of Spontaneously Hypertensive Rats

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Summary. The effect of a traditional Chinese medicine, Toki-shakuyaku-san (Dang-Gui-Shao-Yao-san), on placental blood flow was evaluated in spontaneously hypertensive rats (SHR). Pregnant SHR and Wistar-Kyoto rats (WKY) which were divided into 6 groups, were administered either water or 1.5% saline. The rats in the SHR+drug group and the SHR+saline+drug group were administered daily doses of 800mg/Kg of Toki-shakuyaku-san. No drug was administered to the WKY, SHR, WKY+saline and SHR+saline groups. Systolic blood pressure was measured on days 0, 5, 10, 15, 20 of pregnancy using the tail cuff method. The placental blood flow on the 20th day of pregnancy was determined using the electrolytic hydrogen clearance method.

The placental blood flow (46 ± 4 vs. 40 ± 5 ml/min/100g, $p < 0.05$) and fetal body weight (3.51 ± 0.08 vs. 3.46 ± 0.06 g, $p < 0.05$) in the SHR+saline+drug group were higher than those in the SHR+saline group, while there was no change in blood pressure. It is suggested that one of the etiologies of intrauterine growth retardation of the fetus is a decrease in the placental blood flow, and that Toki-shakuyaku-san accelerated development of the fetus by improving the placental blood flow.

INTRODUCTION

Although much is unclear about the pathogenesis of toxemia in pregnancy, it has been believed in Western medicine that the major factors of this disease were systemic vasospasms and decreased uteroplacental blood flow.¹⁻³ The disease is composed of three major symptoms, edema, proteinuria and hypertension. In contrast, traditional Chinese medicine has paid attention to a deficiency of the spleen and kidney, i.e., that disorders of the body's water metabolism are induced by water stagnation owing

to the weakening of the digestive tract function and diencephalon-pituitary-adrenal-ovarian function.⁴

On the other hand, small for date baby (SFD) is currently an important topic in perinatal medicine. This occurs frequently in cases of birth after pregnancies that involved toxemia.^{1-3,5} The prognosis for cases in which placental dysfunction is found tends to be poor.^{6,7} Therefore, not only dietary therapy, but also diuretics, hypotensive agents and others are used in cases of severe toxemia in pregnancy.

Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San) is a traditional Chinese medicine that has been used in the past against toxemia in pregnancy. The drug has the effect of overcoming blood stagnation and diuresis.^{4,8} However, few reports are available proving its effects. This has been due, in particular, to the difficulty in measuring the placental blood flow in humans.^{5,9-11}

In the present study, we used spontaneously hypertensive rats (SHR) as model animals of toxemia in pregnancy. We studied the effects of Toki-shakuyaku-san on the blood pressure, fetal weight and placental blood flow.

MATERIALS AND METHODS

Nulliparous 12-week-old female SHR and Wistar-Kyoto rats (WKY) from Charles River Co. were used as the experimental models. They were reared in a completely air-conditioned environment ($22 \pm 2^\circ\text{C}$, $50 \pm 10\%$ humidity) with light from 6:00 am to 6:00 pm. Solid feed was given at liberty. The animals were divided into groups consisting of a WKY group and an SHR group to which water was administered, a WKY+saline and an SHR+saline group to which

1.5% saline was administered, and an SHR+drug group and an SHR+saline+drug group in which Toki-shakuyaku-sun was administered. The day on which sperms were found in the vaginal smear of females was taken as day 0 of pregnancy.

Although there were to be twelve of each type of rat in each group, the pregnant rats were 92% (22/24) in WKY and 71% (34/48) in SHR. At the end, there were 11 animals in the WKY group, 11 in the WKY+saline group, 8 in the SHR group, 10 in the SHR+saline group, 7 in the SHR+drug group and 9 in the SHR+saline+drug group. The drug was administered daily from day 1 to day 20 of pregnancy using a special water supply bottle so as to be 800mg/Kg/day of Toki-shakuyaku-san extract powder (Tsumura Co.).

The systolic pressure was measured on days 0, 5, 10, 15 and 20 of pregnancy by the tail cuff method, using a caudal artery blood pressure recording apparatus (Riken Kaihatsu Co., PS-100). The mean values of three measurements were determined.

The animals were subjected to laparotomy under ether anesthesia on day 20 of pregnancy and the placental blood flow was measured by one electrode using an electrolytic hydrogen clearance tissue rheometer (Biomedical Science Co., RBF-222).^{12,13)}

The placental blood flow was measured by connecting it to a data analysis computer (Epson Co., HC-40). Measurements were conducted with an electrolysis current of 20 μ A and an electrolysis time of 25 sec. The hydrogen gas concentration in the tissue after electrolysis was completed was measured 300 times at one-second intervals and a hydrogen clearance curve was drawn by the data analysis device. The half-life was determined therefrom and the tissue blood flow was determined. The spontaneous diffusion rate of hydrogen was determined to be 20 ml/min/100g.

Statistical analysis was conducted by the Student t-test and the analysis shown as the mean \pm 1SD with $p < 0.05$ being taken as significant.

RESULTS

1. Number of fetuses per dam (Table 1)

The number of fetuses per dam tended to be fewer in the SHR groups than in the WKY groups. When saline was administered a significant decrease occurred ($p < 0.05$). Although drug administration tended to increase the number, no significant difference could be seen.

2. Weight per fetus (Table 2)

The weight was lower in the SHR groups in comparison to the WKY groups ($p < 0.05$). Drug administration tended to increase weight. Although the weight showed no change in the WKY+saline group compared to the WKY group, the weight was less in the SHR+saline group than in the SHR group ($p < 0.05$). A significant increase was seen in the weight of the SHR+saline+drug group in comparison to the SHR+saline group ($p < 0.05$).

When the mean minus 1SD of the fetal weight of the SHR groups was taken to determine SFD, SFD

Table 1. Number of fetuses per dam

WKY (n=11)	SHR (n=8)	SHR+drug (n=7)
11.2 \pm 3.2	8.4 \pm 3.6	7.1 \pm 3.4
WKY+saline (n=11)	SHR+saline (n=10)	SHR+saline+drug (n=9)
10.0 \pm 2.8	6.2 \pm 3.4*	7.1 \pm 3.6

* $p < 0.05$ vs WKY+saline. Values are mean \pm 1SD.

Table 2. Weight per fetus (g)

WKY	SHR	SHR+drug
3.58 \pm 0.09	3.51 \pm 0.08	3.56 \pm 0.08
p < 0.05		NS
NS		
WKY+saline	SHR+saline	SHR+saline+drug
3.59 \pm 0.09	3.46 \pm 0.06*	3.51 \pm 0.08
p < 0.01		p < 0.05
p < 0.01		

* $p < 0.05$ vs SHR.

was found in 15% in the SHR group, 5% in the SHR+drug group, 65% of the SHR+saline group and 15% of the SHR+saline+drug group. The development of SFD was increased by saline loading and decreased by drug administration.

3. Changes in blood pressure (Fig. 1)

The blood pressure dropped at the end of pregnancy in WKY, SHR and SHR+drug groups. No

difference could be recognized between the SHR group and the SHR+drug group.

Although no difference could be seen between the WKY group and the WKY+saline group, the SHR+saline group tended to be higher in comparison to the SHR group. It was significantly higher on day 20 of pregnancy ($p < 0.05$).

No difference was recognized between the SHR+

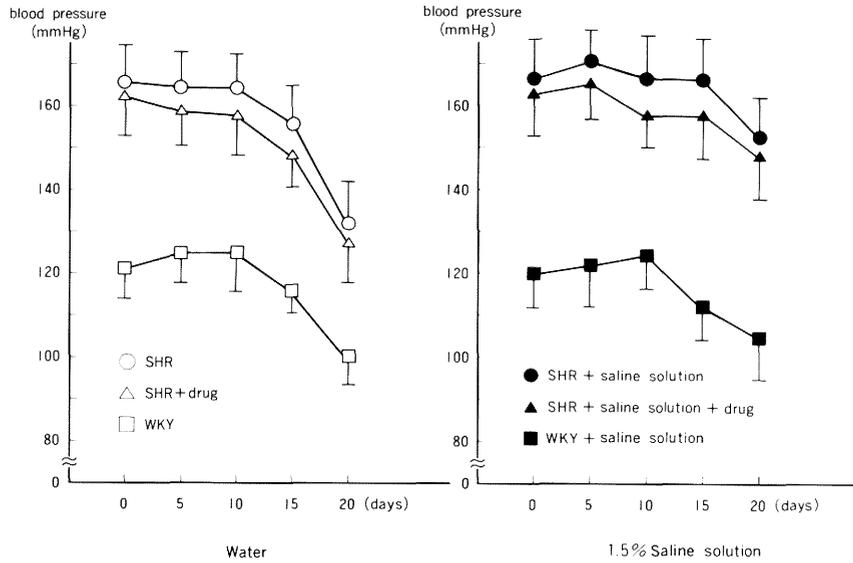


Fig. 1. Changes in the maternal blood pressure during pregnancy. The effect of Toki-shakuyaku-san on the systolic blood pressure in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY).

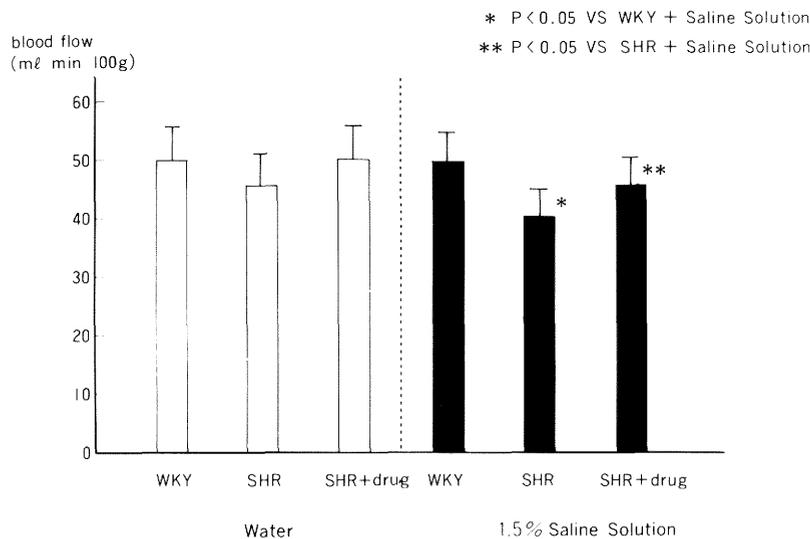


Fig. 2. The effect of Toki-shakuyaku-san on the placental blood flow.

saline group and the SHR+saline+drug group. The blood pressure was not decreased even by drug administration.

4. Placental blood flow (Fig. 2)

The blood flow tended to be lower in the SHR groups than in the WKY groups. Drug administration tended to increase blood flow. This tendency was conspicuous under saline administration. While the WKY+saline group did not differ from the WKY group, the SHR+saline group was markedly lower in comparison to the SHR group ($p < 0.05$).

The SHR+saline+drug group was significantly higher in comparison to the SHR+saline group ($p < 0.05$).

DISCUSSION

The fetal body weight was lower in SHR than in WKY. Decreased placental blood flow was believed to be one of the mechanisms of the onset of SFD. Similarly, SFD occurs in toxemia in pregnancy.^{6,7,14-16} Increasing the placental blood flow without negatively affecting the mother and fetus is believed to be one means of preventing the development of SFD.^{14-16,17} On the other hand, salt is closely related to the development of hypertension and adversely affects preventive treatment of toxemia in pregnancy. Salt load increases SFD and intrauterine fetal death.¹⁸ Pregnant SHR with a salt load was used as a model of human toxemia in pregnancy based on the above and the effects of Toki-shakuyaku-san on the blood pressure, fetal body weight and placental blood flow were studied.

Although administration of Toki-shakuyaku-san did not clearly decrease the blood pressure, it increased the fetal weight and increases were also in the placental blood flow. It seems therefore reasonable to suggest that Toki-shakuyaku-san administration improves fetal development in toxemia in pregnancy.

Drug treatment in Western medicine has frequently posed problems for society such as side effects and, especially, fetal teratogenicity.³ Pregnant women particularly tend to be hypersensitive to drugs. In contrast, traditional Chinese medicines, which have been used for some thousands of years, are said to be almost free of side effects.^{4,19}

Toki-shakuyaku-san acts to decrease the blood viscosity, improve blood circulation and ameliorate water stagnation.^{4,20,21} It is made of 6 crude drugs; Angelicae Radix, Paeoniae Radix, Cnidii Rhizoma, Hoelen, Atractylodis Lanceae Rhizoma and Alismatis Rhizoma.^{4,19,20,22} Angelicae Radix, Paeoniae

Radix and Cnidii Rhizoma have the effects of warming and overcoming blood stagnation. Paeoniae Radix, Cnidii Rhizoma and Atractylodis Lanceae Rhizoma have sedative effects. Atractylodis Lanceae Rhizoma, Hoelen and Alismatis Rhizoma have diuretic effects. Angelicae Radix, Paeoniae Radix and Atractylodis Lanceae Rhizoma stabilize placental function.^{22,23} In Japan, this drug is used to treat various obstetrical diseases, including toxemia in pregnancy.

Two factors have been suggested to be involved in the pathologic condition of toxemia in pregnancy: decreased uteroplacental blood flow and systemic vasospasm. Toki-shakuyaku-san is believed to improve at least one of these two factors. Further clinical studies are necessary to clarify the mechanisms of the effects of this traditional Chinese medicine.

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