Renal function-improving effect of Salviae Miltiorrhizae Radix extract

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(Received February 25, 1987. Accepted May 1, 1987.)

Abstract

The acute effect of Salviae Miltiorrhizae Radix extract on renal function was investigated in renal failure rats induced by an adenine diet. Renal function studies were performed at 5.5 to 6.0 hr after injecting a single 10 mg/100 g of body weight intraperitoneal dose of the Salviae Miltiorrhizae Radix extract. Glomerular filtration rate (GFR), renal plasma flow (RPF), and renal blood flow (RBF) progressively decreased as renal impairment increased due to extended administration of adenine. Treatment with the extract significantly increased GFR by 52%, 50%, and 32% in renal failure rats administered with adenine on the 6th, 12th, and 18th days, respectively. RPF increased by 41% and 40% on the 6th and 12th days, respectively. RBF increased by 41% and 37% on the 6th and 12th days, respectively. However, there were no effects of the extract on rats who have renal failure with severe renal impairment, as shown on the 24th and 30th days. In filtration fraction (FF), no significant changes were observed throughout the experiment. These data suggest that Salviae Miltiorrhizae Radix is an effective herb when given as therapy for the treatment of mild to moderate renal failure.

Key words Salviae Miltiorrhizae Radix, glomerular filtration rate, renal plasma flow, renal blood flow, renal failure rat

Abbreviations GFR, glomerular filtration rate ; RPF, renal plasma flow ; Ht, hematocrit value ; RBF, renal blood flow ; FF, filtration fraction

Introduction

Salviae Miltiorrhizae Radix, a well-known traditional Chinese medicinal herb, was experientially used to improve blood circulation, relieve blood stasis, eliminate swellings, *etc*.[1] In addition, it has recently been reported to show vasodilative, hypotensive, anticoagulant, and antibacterial activities, and to have a beneficial effect in patients with chronic renal failure.[2,3] However, its effect on renal function has not been well investigated. Therefore, in our laboratory, a series of experiments have been conducted to elucidate the renal effects of the extract from this herb.

Our previous report showed that the extract from Salviae Miltiorrhizae Radix increased urinary excretions of urea and creatinine after a single intraperitoneal administration in normal rats, which indicated an improvement of renal function. Treatment with the extract also showed natriuretic, kaliuretic, and phosphaturic responses.[4] The acute effect of this extract on urinary excretion was investigated in renal failure rats induced by an adenine diet. Significant increases in urinary urea, creatinine, sodium, and inorganic phosphate excretions were observed in cases of mild kidney damage.[5]

In the present paper, further studies were carried out to investigate the effect of the Salviae Miltiorrhizae Radix extract on renal function of

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Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 4, 59－64, 1987

renal failure rats, in order to reveal whether treatment with the extract could have a beneficial effect on renal failure. These studies with experimental renal failure animals were also carried out to elucidate which stage of renal failure the extract was effective in.

Materials and Methods

Animals and treatment : Male rats of the LWH : Wistar strain with a body weight of 200–210 g, were placed in metabolic cages under a temperature of 25±1℃ and a 12 hr dark-light cycle. They were allowed an adaptation period of 10 days and fed on a commercial feed (CLEA Japan Inc., Tokyo, Japan, type CE-2) during the adaptation period. Then they were fed *ad libitum* on an 18% casein diet containing 0.75% adenine, which produced rats with renal failure experimentally. In the rats with renal failure induced by adenine, renal impairment become aggravated by increasing the days of adenine feeding. Azotemia, an abnormal urea cycle, abnormal pattern of free amino acids in the blood, and abnormal metabolism of calcium and phosphorus have previously been observed.[6-9] Pathological studies of the kidneys of such animals have revealed lesions of the proximal tubules, of some distal tubules and of glomeruli.[8,9] Administration of the adenine diet for 6, 12, 18, 24, or 30 days was followed by administration of the Salviae Miltiorrhizae Radix extract (10 mg/100 g of body weight) made soluble in saline, and administered intraperitoneally to the rats. Control rats were treated with an equal volume of saline.

Preparation of extract from Salviae Miltiorrhizae Radix : The roots of Salviae Miltiorrhizae Radix (*Salvia Miltiorrhiza* BUNGE) grown in China, and supplied by Tochimoto Tenkaido Co., Ltd., Osaka, Japan, were finely powdered and extracted with distilled water at 100℃ for 40 min (roots : water=1 : 10, w/v), as described previously.[10] The aqueous extract was then filtered through 4 layers of gauze and the filtrate was freeze-dried under reduced pressure to provide a brown residue of about 25% yield.

Renal function study : Glomerular filtration rate (GFR), renal plasma flow (RPF), hematocrit value (Ht), and renal blood flow (RBF) values were obtained at 5.5 to 6.0 hr after intraperitoneal administration of the Salviae Miltiorrhizae Radix extract. GFR and RPF were measured by means of renal clearance test using a single intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as indicator.[11,12] At 25 min after intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, the bladder was reflexly emptied by having each rat inhale ether for 3–5 sec. The urine thus voided was discarded. During the next 30 min, the urine was collected, which was terminated after the bladders were again emptied reflexly by ether inhalation. Blood samples were taken from conscious rats by heart puncture at the middle of the period for the clearance test. Thiosulfate and *para*-aminohippurate were determined by titrimetry and colorimetry, respectively. RBF was calculated on the basis of RPF and Ht using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

$$RBF=\frac{RPF}{1-Ht}(ml/min)$$

Statistics : The significance of differences between the control and Salviae Miltiorrhizae Radix extract-treated groups was tested by the use of Student's t-test. A p value greater than 0.05 was considered not to be statistically significant.

Results

Effect of Salviae Miltiorrhizae Radix extract on GFR

Changes in GFR were shown in Fig. 1. GFR in normal rats was 5.65±0.27 ml/min/kg. The GFR progressively dropped as kidney damage increased due to extended administration of adenine. The GFR decreased markedly by 51% on day 6, by 61% on day 12, by 80% on day 18, by 93% on day 24, and 96% on day 30, in comparison with normal value (on day 0). Treatment with the Salviae Miltiorrhizae Radix extract signifi-

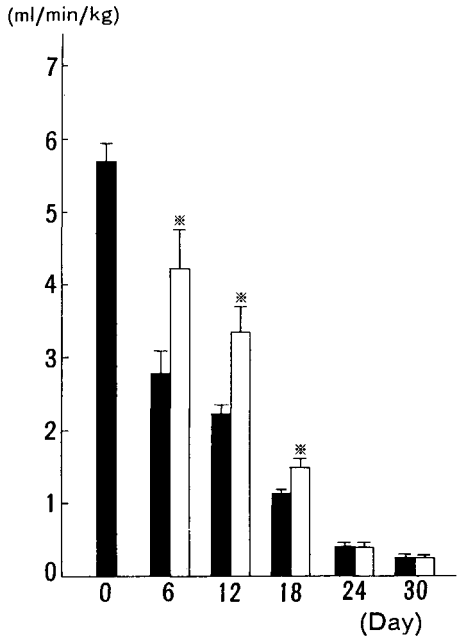

Fig. 1 Effect of Salviae Miltiorrhizae Radix extract on glomerular filtration rate.
■, control group ; □, Salviae Miltiorrhizae Radix extract-treated group. Values are means ±S.E. of 6 rats. ※ Significantly different from the control value, $p<0.05$.

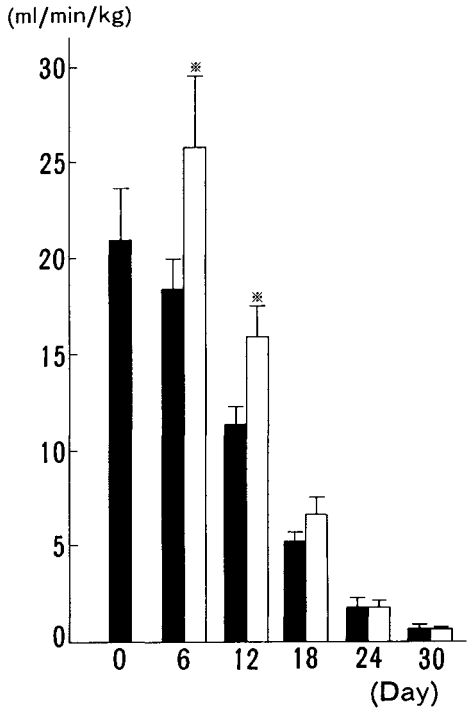

Fig. 2 Effect of Salviae Miltiorrhizae Radix extract on renal plasma flow.
Details are the same as in the legend to Fig. 1.

cantly increased the GFR on days 6−18 : from 2.76 ml/min/kg to 4.20 ml/min/kg on the 6th day (a 52% change, $p<0.05$), from 2.22 ml/min/kg to 3.33 ml/min/kg on the 12th day (a 50% change, $p<0.05$), and from 1.12 ml/min/kg to 1.48 ml/min/kg on the 18th day (a 32% change, $p<0.05$), respectively. On the contrary, the GFR remained nearly unchanged after the administration of the extract on the 24th and 30th days of adenine diet when renal impairment was severe.

Effect of Salviae Miltiorrhizae Radix extract on RPF

Figure 2 shows the effect of Salviae Miltiorrhizae Radix extract on RPF. Adenine administration induced a gradual fall in RPF as the damage of kidney increased due to sustained administration of it. A fall occurred significantly in RPF by 46%, 75%, 92%, and 97% on days 12, 18, 24, and 30, respectively, compared with normal value. However, RPF of the Salviae Miltiorrhizae Radix extract-treated group in renal failure rats was significantly higher than that of the control group. As shown in Fig. 2, Salviae Miltiorrhizae Radix extract resulted in a significant rise in RPF : from 18.31 ml/min/kg to 25.76 ml/min/kg (a 41% change, $p<0.05$) on day 6 and from 11.32 ml/min/kg to 15.80 ml/min/kg (a 40% change, $p<0.05$) on day 12. On day 18, RPF showed a tendency to increase from 5.11 ml/min/kg to 6.48 ml/min/kg (a 27% change) after treatment with the extract.

Effect of Salviae Miltiorrhizae Radix extract on filtration fraction (FF)

As shown in Table I, the FF (GFR/RPF) decreased significantly by 40% and 20% on days 6 and 12, respectively and tended to decrease by 12% on day 18. The FF increased by 16% on day 24 (not significant) and significantly by 48% on day 30. Treatment with the Salviae Miltiorrhizae Radix extract slightly elevated FF on days 6,

Table I Effect of Salviae Miltiorrhizae Radix extract on filtration fraction.

Day	Group	FF
0	–	0.25±0.02
6	Control	0.15±0.01
	Salviae Miltiorrhizae Radix extract	0.17±0.01
12	Control	0.20±0.01
	Salviae Miltiorrhizae Radix extract	0.23±0.05
18	Control	0.22±0.02
	Salviae Miltiorrhizae Radix extract	0.24±0.02
24	Control	0.29±0.04
	Salviae Miltiorrhizae Radix extract	0.26±0.05
30	Control	0.37±0.05
	Salviae Miltiorrhizae Radix extract	0.44±0.12

Values are means±S.E. of 6 rats.

12, 18, 30, and lowered FF on day 24, compared with control value ; however, these variations were not statistically significant.

Effect of Salviae Miltiorrhizae Radix extract on RBF

RBF calculated from RPF and Ht, decreased together with increase in renal impairment due to extended administration of adenine, as shown in Fig. 3. The RBF decreased significantly by 51%, 78%, 93%, and 98% on days 12, 18, 24, and 30, respectively, compared with normal value. Effect of Salviae Miltiorrhizae Radix extract on RBF was nearly parallel to RPF seen in Fig. 2 because Ht remained nearly unchanged when compared with control value. However, administration of adenine diet was followed by a marked decrease in Ht ; Ht fell significantly by 20%, 19%, 25%, and 36%, on days 12, 18, 24, and 30, respectively, when compared with normal value (Table II). Thus, the extract treatment significantly increased RBF in renal failure rats : from 33.30 ml/min/kg to 46.96 ml/min/kg (a 41% change, $p<0.05$) and from 17.18 ml/min/kg to 23.50 ml/min/kg (a 37% change, $p<0.05$) on days 6 and 12, respectively. On day 18, the extract tended to increase RBF from 7.60 ml/min/kg to 9.69 ml/min/kg, a 28% change. However, on

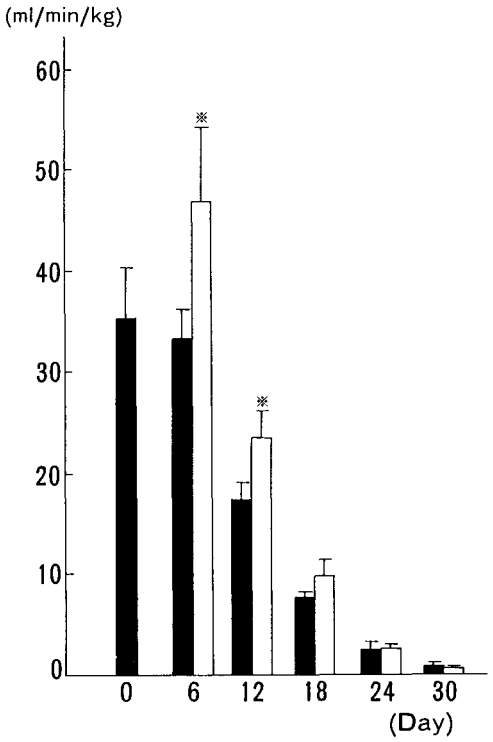

Fig. 3 Effect of Salviae Miltiorrhizae Radix extract on renal blood flow.
Details are the same as in the legend to Fig. 1.

Table II Effect of Salviae Miltiorrhizae Radix extract on hematocrit value.

Day	Group	Ht (%)
0	–	40.6±0.8
6	Control	41.1±1.4
	Salviae Miltiorrhizae Radix extract	40.9±1.2
12	Control	32.6±1.3
	Salviae Miltiorrhizae Radix extract	32.4±1.3
18	Control	33.0±1.9
	Salviae Miltiorrhizae Radix extract	31.8±1.7
24	Control	30.4±1.2
	Salviae Miltiorrhizae Radix extract	31.7±0.8
30	Control	26.0±1.3
	Salviae Miltiorrhizae Radix extract	28.0±1.0

Values are means±S.E. of 6 rats.

days 24 and 30, there were no significant differences in RBF between the control and the Salviae Miltiorrhizae Radix extract-treated groups.

Discussion

In chronic renal diseases associated with a gradual reduction in the number of functioning nephrons, the decreases of the filtration area of glomerular capillaries and of blood supply due to circulatory insufficiency cause decreases in GFR and RBF.[13]

The present experimental results in the rats with renal failure induced by adenine also show that GFR and RBF decreased as kidney damage increased due to sustained administration of adenine. The intraperitoneal administration of Salviae Miltiorrhizae Radix extract markedly increased GFR on days 6, 12, and 18, and RBF on days 6 and 12 as shown in Figs.1 and 3, whereas these same parameters were not altered significantly on days 24 and 30 of adenine administration. These observations suggest that renal failure rats with mild to moderate renal impairment have reversible alteration in renal vasculature that is modulated, in part, by the treatment with Salviae Miltiorrhizae Radix extract.

Treatment with the Salviae Miltiorrhizae Radix extract caused no significant alterations in FF, but increased GFR and RPF. GFR primarily depends on the mean transcapillary hydraulic pressure difference, which is thought to be altered by changes in either afferent or efferent arteriolar tone.[14] Further studies need to be performed in order to evaluate the effect of this extract on the dilatation of the afferent or efferent arteriole.

On the other hand, a high degree of dependence of GFR on RPF was recently reported, *i.e.*, the increases in plasma flow are accompanied by proportional increase in GFR. In studies by Brenner *et al*.,[15] a direct relationship between GFR and RPF was reported to follow vasodilation. However, no dependency of GFR on RPF was reported by Stein *et al*.[16] Significant increase in RPF in the dog following vasodilation with acetylcholine or bradykinin has little or no effect on the simultaneously measured filtration rate. Thus, an increase in RPF has no effect on filtration. In the present study, the GFR increased with a concomitant increase in RPF. These results supported the experimental results reported by Brenner *et al*.[15] that the changes in RPF had an effect on filtration rate.

In the previous report,[5] it was shown that Salviae Miltiorrhizae Radix extract significantly increased urine volume and urinary excretion of substances known to reflect changes in renal function and its effects were different in the various stages of renal failure. Based on the experimental results of the present study, one of the reasons for this is considered to be the increase of GFR, RPF, and RBF. However, there were no effects of the extract in renal failure rats administered with adenine on the 24th and 30th days. The real reason for these phenomena remains undefined. In rats fed on an adenine diet, the serum levels of urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid, *etc.* increased according to the number of days of adenine feeding.[6-9] Pathological studies of the kidneys in such animals have revealed lesions of the proximal tubules, a proportion of the distal tubules and the glomeruli.[6,8,9] Thus, it is possible that Salviae Miltiorrhizae Radix extract might ameliorate the mild to moderate stages of renal failure by facilitating renal function, when remnant nephrons are still functioning to some extent, as shown on the 6th and 12th days of adenine administration. Studies on the effect of Salviae Miltiorrhizae Radix using a 70% nephrectomized rats have been carried out by Li *et al*.[17] ; this report supports our studies.

Considering the results from these studies using the extract from Salviae Miltiorrhizae Radix, this crude drug might be expected to cause alterations in renal function by several possible mechanisms, including changes in the transcapillary hydraulic and oncotic pressures, hemodynamics, direct effects on renal tubular sodium reabsorption, and changes in humoral factors such as renin, angiotensin, aldosterone, vasopressin, prostaglandin, catecholamine, *etc.* that affect renal function. Although further studies need to be performed in order to define the precise mode of action of the Salviae Miltiorrhizae Radix extract, we conclude that this crude drug is an effective herb when given as therapy for the treatment of mild to moderate renal failure.

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