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Acute Effect of Extract from Salviae Miltiorrhizae Radix on Renal Function in Renal Failure Rats

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The acute effect of Salviae miltiorrhizae Radix extract on renal function was investigated in rats with renal failure induced by an adenine diet. 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 50% in renal failure rats on the 12th d of adenine administration. The RPF in the extract-treated group also increased by 40% as compared with the control group. Similarly, Salviae miltiorrhizae Radix extract significantly increased RBF by 37%. However, the extract was ineffective in rats having renal failure with severe renal impairment, on the 24th d of adenine administration. On the other hand, the urinary excretions of urea and creatinine increased significantly by 27% and 19%, respectively, on the 12th d. These data suggest that Salviae miltiorrhizae Radix is an effective herb for the treatment of mild renal failure.

Keywords——Salviae miltiorrhizae Radix; glomerular filtration rate; renal plasma flow; renal blood flow; urea; creatinine; renal failure rat

Salviae miltiorrhizae Radix, a well-known traditional medicinal herb, has been experientially shown to improve blood circulation, relieve blood stasis, and eliminate swellings.[1] It was 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] 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; this result indicated an improvement of renal function. Treatment with the extract also showed natriuretic, kaliuretic, and phosphaturic responses.[4] The purpose of the present study was to determine whether Salviae miltiorrhizae Radix could produce an elevation of renal function in rats with renal failure and have a beneficial effect on renal failure. These studies with experimental renal failure animals were also carried out to ascertain which stage of renal failure the extract was effective in.

Materials and Methods

Animals and Treatments——Male rats of the LWH: Wistar strain, with a body weight of 200—210 g, were placed in metabolic cages at a temperature of 23 ± 1 °C and a 12 h dark—light cycle. They were allowed an adaptation period of 10 d 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 experimental renal failure. In the rats with renal failure induced by adenine, renal impairment becomes aggravated as the time of adenine feeding is increased. Findings of 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.[5-8] On the 12th day of adenine feeding, glomerular filtration rate (GFR) and renal blood flow (RBF) were one-third to one-half of the normal values, and renal histologic changes including degeneration of tubular cells and dilation of the

tubulus were observed. When the adenine diet was given for 24 d in order to induce a more severe state of renal failure, GFR and RBF were about one-tenth of the normal values, and foreign body granuloma was formed in the renal tubules and interstitium. Marked fibrosis leading in some extreme cases to contracted kidney and a slight decrease in the number of glomeruli were also noted.[5,7-9] The present experiment was carried out in rats fed on an adenine diet for 12 or 24 d, in order to elucidate whether the effect of Salviae miltiorrhizae Radix extract is dependent on the degree of renal damage or not. In the preliminary experiment, the intraperitoneal administration of Salviae miltiorrhizae Radix extract dose-dependently decreased the serum creatinine level in renal failure rats. Thus, Salviae miltiorrhizae Radix extract (10 mg/100 g of body weight) in saline was 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 °C 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 in about 25% yield.

Sample Collection and Analyses——In the preliminary experiment, urea nitrogen and creatinine in the serum decreased most at 6 h after intraperitoneal administration of the Salviae miltiorrhizae Radix extract. Urinary urea and creatinine excretions were highest at 3—6 h after intraperitoneal treatment with the extract. GFR, renal plasma flow (RPF), hematocrit value (Ht), RBF, urea, and creatinine were examined at 5.5 to 6.0 h after intraperitoneal administration of the Salviae miltiorrhizae Radix extract. GFR and RPF were measured by means of a renal clearance test using a single intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as an 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 s. The urine thus voided was discarded. During the next 30 min, the urine was collected, and at the end of this period, the bladder was again emptied reflexly by ether inhalation (this urine formed part of the collected sample). 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). The urinary urea and creatinine were estimated in the urine obtained in the 30 min collection period described above. Urea was determined by the Archibald method.[13] Creatinine was

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

determined by the Folin–Wu method,[14] with a commercial reagent (Creatinine-Test Wako) supplied by Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Statistics——Results were expressed as means ± S.E. of 6 rats. 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 significant.

Results

Effect of Salviae Miltiorrhizae Radix Extract on Clearance of Thiosulfate

As shown in Table I, the GFR calculated on the basis of the plasma and urinary thiosulfate levels progressively dropped as kidney damage increased due to extended administration of adenine. The GFR decreased markedly by 61% on day 12 and 93% on day 24, in comparison with the normal value (on day 0). Treatment with the Salviae miltiorrhizae Radix extract significantly increased the GFR on day 12: from 2.22 to 3.33 ml/min/kg on the 12th d (a 50% change, $p < 0.05$), which was calculated from the decreased plasma and the increased urinary levels of thiosulfate. On the other hand, the plasma and urinary thiosulfate levels, and GFR remained nearly unchanged after the administration of the extract on the 24th d of adenine diet, when renal impairment was severe.

Effect of Salviae Miltiorrhizae Radix Extract on Clearance of *para*-Aminohippurate, Ht, and RBF

Table II shows the effect of Salviae miltiorrhizae Radix extract on the clearance of *para*-aminohippurate. Adenine administration induced a greater fall in clearance of *para*-aminohippurate on day 24 when the damage to the kidneys had increased due to the sustained administration, compared with that on day 12. The extract tended to decrease plasma *para*-

Table I. Effect of Salviae Miltiorrhizae Radix Extract on Clearance of Thiosulfate

Day	Group	p-Thio (mg/dl)	u-Thio (mg/30 min)	GFR (ml/min/kg)
0	—	18.90±1.20	8.21±0.36	5.65±0.27
12	Control	26.22±3.44	3.44±0.30	2.22±0.12
	Salviae miltiorrhizae Radix extract	20.13±2.93	4.29±0.37	3.33±0.36[a]
24	Control	23.71±1.10	0.57±0.08	0.40±0.07
	Salviae miltiorrhizae Radix extract	25.87±1.52	0.63±0.07	0.40±0.06

Abbreviations: p-Thio, plasma thiosulfate; u-Thio, urinary thiosulfate; GFR, glomerular filtration rate. *a*) Significantly different from the control value, $p<0.05$.

Table II. Effect of Salviae Miltiorrhizae Radix Extract on Clearance of *para*-Aminohippurate, Hematocrit Value, and Renal Blood Flow

Day	Group	p-PAH (mg/dl)	u-PAH (mg/30 min)	RPF (ml/min/kg)	Ht (%)	RBF (ml/min/kg)
0	—	2.78±0.18	4.78±0.85	20.83±2.73	40.6±0.8	35.31±5.03
12	Control	3.09±0.32	2.06±0.06	11.32±0.91	32.6±1.3	17.18±1.74
	Salviae miltiorrhizae Radix extract	2.64±0.44	2.37±0.24	15.80±1.59[a]	32.4±1.3	23.50±2.61[a]
24	Control	2.77±0.15	0.26±0.06	1.69±0.53	30.4±1.2	2.43±0.78
	Salviae miltiorrhizae Radix extract	2.83±0.25	0.28±0.04	1.73±0.36	31.7±0.8	2.52±0.50

Abbreviations: p-PAH, plasma *para*-aminohippurate; u-PAH, urinary *para*-aminohippurate, RPF, renal plasma flow; Ht, hematocrit value; RBF, renal blood flow. *a*) Significantly different from the control value, $p<0.05$.

aminohippurate but to increase urinary *para*-aminohippurate on day 12. Thus, the extract resulted in a significant rise in RPF, calculated from the plasma and urinary levels of *para*-aminohippurate: from 11.32 to 15.80 ml/min/kg (a 40% change, $p<0.05$) on day 12. No change in clearance of *para*-aminohippurate was observed after the administration of the extract on the 24th d of adenine diet.

Administration of adenine diet was also followed by a marked decrease in Ht, which fell significantly on days 12 and 24 compared with the normal value. However, the extract treatment remained nearly unchanged when compared with the control group (Table II).

RBF, calculated from RPF and Ht, decreased with increase in renal impairment due to extended administration of adenine, as shown in Table II. The RBF on day 24 decreased significantly by 93% compared with the normal value. The effect of Salviae miltiorrhizae Radix extract on RBF was nearly parallel to that on RPF because Ht remained approximately unchanged when compared with the control group. Namely, the treatment with the extract significantly increased RBF on day 12 in renal failure rats: from 17.18 to 23.50 ml/min/kg (a 37% change, $p<0.05$). There was no significant difference in RBF between the control and extract-treated groups on day 24.

Effect of Salviae Miltiorrhizae Radix Extract on Urinary Excretions of Urea and Creatinine

As shown in Table III, urinary excretions of urea and creatinine were decreased as renal impairment increased due to extended administration of adenine. However, treatment with Salviae miltiorrhizae Radix extract significantly increased urinary excretion of urea: from 1.47 to 1.86 mg/min/kg (a 27% change, $p<0.05$). On day 24, Salviae miltiorrhizae Radix extract showed a tendency to increase the urinary urea excretion (by 18%). In addition,

TABLE III. Effect of Salviae Miltiorrhizae Radix Extract on Urinary Excretions of Urea and Creatinine

Day	Group	Urea (mg/min/kg)	Creatinine (μg/min/kg)
0	—	2.75±0.27	42.4±5.4
12	Control	1.47±0.09	26.3±1.2
	Salviae miltiorrhizae Radix extract	1.86±0.18[a)]	31.2±2.0[a)]
24	Control	1.25±0.14	19.9±1.0
	Salviae miltiorrhizae Radix extract	1.47±0.15	21.7±1.4

a) Significantly different from the control value, $p<0.05$.

treatment with the extract on the 12th day significantly increased urinary creatinine excretion: from 26.3 to 31.2 μg/min/kg (a 19% change, $p<0.05$). On day 24, the extract tended to increase urinary creatinine excretion (by 9%).

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 the blood supply due to circulatory insufficiency cause decreases in GFR and RBF.[15)]

The present results in 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 and RBF on the 12th d of adenine feeding as shown in Tables I and II, whereas these parameters were not altered significantly on the 24th d. The changes in GFR and RBF were more remarkable in the rats with mild renal failure than in the rats with severe renal failure. Therefore, the increase of GFR and RBF after treatment with the extract seemed to be characteristic of the stage of renal failure in rats. These observations suggest that renal failure rats with mild renal impairment have a reversible alteration in renal vasculature that is modulated, in part, by the treatment with Salviae miltiorrhizae Radix extract.

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 with proportional increases in GFR. In studies by Brenner *et al.*,[16)] a direct relationship between GFR and RPF was reported to follow vasodilation. In the present study, treatment with the Salviae miltiorrhizae Radix extract caused no significant alteration in filtration fraction (FF = GFR/RPF) (in general, FF decreases in cases of glomerular disease, is mostly normal in tubular or interstitial disease, and tends to increase in vascular renal diseases[17)]), but increased GFR together with RPF. These results are consistent with the experimental result reported by Brenner *et al.*[16)] that the changes in RPF had an effect on filtration rate. However, 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.[18)] Further studies need to be performed in order to evaluate the effect of this extract on the dilation of the afferent or efferent arterioles.

Salviae miltiorrhizae Radix extract also caused a significant increase in the urinary excretions of urea and creatinine (known to reflect changes in renal function), and these effects were different in the various stages of renal failure. One of the reasons for this is considered to be the increase of GFR, RPF, and RBF. The extract is likely to ameliorate the mild stages of renal failure by facilitating renal function, when remnant nephrons are still functioning to

some extent, as shown on the 12th d of adenine administration.

Although further studies need to be performed in order to define the precise mode of action of the Salviae miltiorrhizae Radix extract, 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, and changes in humoral factors such as renin, angiotensin, aldosterone, vasopressin, prostaglandin, catecholamine, *etc.* that affect renal function. The effects of the extract on renal function observed in renal failure rats in the present study provide a rational basis for the use of this drug in the treatment of renal failure patients with mildly impaired renal function, as reported previously.[3)]

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