Effect of red ginseng powder in rats with chronic renal failure

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Abstract

The effect of oral administration of red ginseng on serum constituents and renal blood flow was determined in rats with chronic renal failure induced by adenine feeding. The rats treated with the ginseng exhibited a reduction in the levels of serum urea nitrogen, creatinine, inorganic phosphate, and calcium. The level of methylguanidine in the serum was significantly decreased at ginseng doses of 7.5 and 15 mg/rat/day. A significant decrease in guanidinosuccinic acid was also observed in the serum of the group given 15 mg ginseng/rat/day. Furthermore, the ginseng-treated groups showed a significant increase in renal tissue blood flow. The improvement of the uremic state by red ginseng is discussed on the basis of the present results.

Key words red ginseng, chronic renal failure, methylguanidine, guanidinosuccinic acid, renal blood flow

Abbreviations GSA, guanidinosuccinic acid ; MG, methylguanidine ; Onpi-tô (Wen-Pi-Tang), 温脾湯

Introduction

The extract from the roots of *Panax ginseng* C. A. MEYER has been used as a therapeutic agent for various diseases including hyperlipemia, atherosclerosis, hypertension, and diabetes mellitus. From the viewpoint of biochemical metabolism, it is known to display a variety of effects of ginseng saponins on lipid and sugar metabolism, as shown by Yamamoto *et al.*,[1-4] Sakakibara *et al.*,[5] Gommori *et al.*,[6] Ikehara *et al.*,[7] and Yokozawa *et al.*[8-11]

On the other hand, ginseng is an important component of the crude drug prescription in "Kanpo" medicine. The empirical use of the ginseng-containing prescription "Onpi-tô (Wen-Pi-Tang)" has recently been initiated in China as a therapy for uremia in patients with chronic renal failure. We[12-14] have previously clarified that Onpi-tô remarkably lowers the levels of serum urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid, and other substances associated with uremic toxemia in rats with chronic renal failure induced by an adenine diet.[15-18] These experimental findings are considered to be indicative of the uremia-improving effect of Onpi-tô clinically. It is thus conceivable that ginseng may have previously unrevealed pharmacological properties, that is, the improvement of body nitrogen imbalance and other factors associated with uremia.

In the present study, an attempt was made to investigate the action of red ginseng powder in

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rats with adenine-induced chronic renal failure in terms of its effect on urea nitrogen, creatinine, electrolytes, and guanidino compounds in serum and blood flow in renal tissue.

Materials and Methods

Animals and treatment : Male rats of the JCL : Wistar strain, initially weighing 110—120 g, were used in this experiment. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, Japan, type CE-2) under a temperature of 25±1°C and a 12-h dark-light rhythm for a week. Then they were fed *ad libitum* on an 18% casein diet containing 0.75 % adenine. The diet had the following composition (in 100 g) : casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[19] 4 g, vitamin mixture[19] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added at a level of 0.75 g/100 g of the diet. The adenine feeding procedure produced experimental chronic renal failure, as reported previously.[15-18] During the adenine feeding period, an aqueous solution of red ginseng powder extracted with distilled water at 100 ℃ for 10 min was administered orally for 24 days to rats as drinking water, while control rats received tap water. Throughout the experimental period, there were no statistically differences between the control and ginseng-treated rats with regard to body weight. The food intake of each rat was essentially proportional to weight change. Six rats were used for each experimental group. Values were expressed as means ± S.E.

Ginseng : Korean red ginseng powder (Lot No. MP 841220) was supplied by Japan Korea Red Ginseng Co., Ltd., Kobe, Japan.

Analyses : On the 24th day of the feeding period, rats were stunned by a sharp blow to the head. Blood was collected in a conical centrifuge tube and the serum was separated by centrifugation immediately after collecting the blood for the determination of urea nitrogen, creatinine, and guanidino compounds. Urea nitrogen was determined using a commercial reagent (BUN KAINOS obtained from Kainos Laboratories, Inc., Tokyo, Japan) based on the urease-indophenol method.[20] Creatinine was determined using a commercial reagent (Creatinine-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the Folin-Wu method.[21] Inorganic phosphate was determined using a commercial reagent (Phosphor B-Test Wako) based on the molybdenum blue method.[22] Calcium was determined using a commercial reagent (Calcium C-Test Wako) based on the orthocresolphthaleic complex compound method.[23] For the determination of methylguanidine and guanidinosuccinic acid levels, serum was deproteinized by addition of TCA (final concentration, 10 %). The supernatant obtained by centrifugation at 3000 rpm for 10 min was injected into a Shimadzu LC-5A liquid chromatograph using a step-gradient system. A fluorescence spectrometer, model RF-540 (excitation 395 nm, emission 500 nm ; Shimadzu Co., Kyoto, Japan) was used for detection of the substances on the column. Renal blood flow was determined with a needle-type bipolar electrode electrolytic organ rheometer (Biomedical Science Co., Ishikawa, Japan), applying a hydrogen gas clearance method.[24,25] The procedure was as follows : rats were anesthetized by intraperitoneal administration of 30 mg/kg body weight of sodium pentobarbital (Abbott Laboratories, North Chicago, Ill., U.S.A.) and laparotomized. Then, a needle-type electrode was obliquely inserted into the renal cortex 1—2 mm below the renicapsule, while plate-type Ag-AgCl electrodes were implanted under the skin. Electrodes A and B were used for the determination of hydrogen concentration and generation of hydrogen gas, respectively. From electrode B a current of 10 μA was applied to the renal cortex for 40 sec, and the change in the polarocurrent of hydrogen gas generated was recorded. The half-life of the approximate index from each figure was then applied to the calculation of renal blood flow using the equation shown below. Apparent blood flow based on the diffusion of hydrogen gas was obtained postmortem from the diffusion data.

$$\frac{69.3}{\text{half-life obtained from clearance curve (min)}} - \text{apparent blood flow based on the diffusion of hydrogen gas in the body (ml/100 g renal tissue/min)}$$

Statistics : The significance of differences between the control and ginseng-treated groups was tested by the use of Student's t-test.

Results

Urea nitrogen, creatinine, inorganic phosphate, and calcium in the serum

The rats of the ginseng-treated group showed a moderate decrease in the level of urea nitrogen in the serum ; as shown in Table I, the value for urea nitrogen was 13 % lower at a ginseng dosage level of 7.5 mg/rat/day as compared with the control group but this was not statistically significant. The creatinine level was also decreased by 10 % as compared with the control upon oral administration of 15 mg/rat/day. However, there were no statistically significant differences between the control and ginseng-treated groups. A slight decrease (11 % compared to the control) was observed in the level of serum inorganic phosphate at a ginseng dose level of 7.5 mg/rat/day. In contrast, the level of serum calcium showed a direct correlation with the amount of ginseng administered to rats ; the administration of 30 mg / rat / day of ginseng significantly decreased it by 14 % of the control value.

Guanidino compounds in the serum

In an examination of the effect of oral administration of red ginseng, it was found that the serum level of guanidino compounds was significantly decreased. As shown in Table II, the

Table I Effect of red ginseng powder on urea nitrogen, creatinine, inorganic phosphate and calcium in the serum.

Material	Dose (mg/rat/day)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	P (mg/dl)	Ca (mg/dl)
Control	—	112.3±16.1 (100)	3.1±0.2 (100)	15.0±1.1 (100)	7.4±0.4 (100)
Ginseng powder	7.5	97.3± 2.1 (87)	3.0±0.2 (97)	13.4±0.6 (89)	6.9±0.4 (93)
Ginseng powder	15	105.0± 8.1 (93)	2.8±0.2 (90)	15.0±0.7 (100)	6.8±0.3 (92)
Ginseng powder	30	124.6± 9.0 (112)	3.2±0.2 (103)	16.3±1.0 (109)	6.4±0.3* (86)

Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$.

Table II Effect of red ginseng powder on levels of guanidino compounds in the serum.

Material	Dose (mg/rat/day)	MG (μg/dl)	GSA (μg/dl)
Control	—	14.1±2.0 (100)	86.1±6.7 (100)
Ginseng powder	7.5	8.8±0.9* (62)	78.8±8.3 (92)
Ginseng powder	15	8.1±1.2* (57)	66.7±5.5* (77)
Ginseng powder	30	11.4±1.1 (81)	93.3±7.3 (108)

MG, methylguanidine ; GSA, guanidinosuccinic acid. Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$.

methylguanidine (MG) level in the serum of the ginseng-treated group was sharply decreased at a dose of 7.5 mg/rat/day. The level of MG was decreased to 8.8 μg/dl on average. A significant decrease was also observed at the 15 mg/rat/day dose level. Further increase in the dose to 30 mg/rat/day did not produce any further decrease in the MG level. In addition, the administration of 15 mg/rat/day of ginseng caused a significant decrease of 23% in the guanidinosuccinic acid (GSA) level. However, there were no statistically significant differences between the control and ginseng-treated groups at the 7.5 mg and 30 mg levels.

Renal blood flow

Figure 1 shows the results of the determination of renal blood flow. Renal blood flow was examined at 24 days after oral administration of 15 mg/rat/day of ginseng. It was recognized that the value in normal rats was 93.1±5.9 ml/100 g tissue/min, but that the adenine-fed rats showed a 42 % decrease of flow as compared with the normal rats. However, the oral administration of 15 mg/rat/day of ginseng caused a significant increase of renal blood flow. Typical clearance curves of the 3 groups are shown in Fig. 2. The decrease of hydrogen concentration in the 3 groups almost approximated an exponential function. The rate of decrease was smaller in the adenine-fed rats.

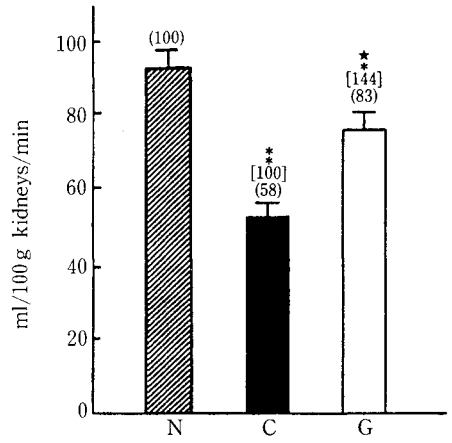

Fig. 1 Blood flow in renal tissue.
N, normal rat ; C, chronic renal failure rat (control group) ; G, chronic renal failure rat (red ginseng-treated group). Figures in parentheses are percentages of the N or C value. *, ★ Significantly different from the N or C value, $p<0.05$, $**p<0.001$.

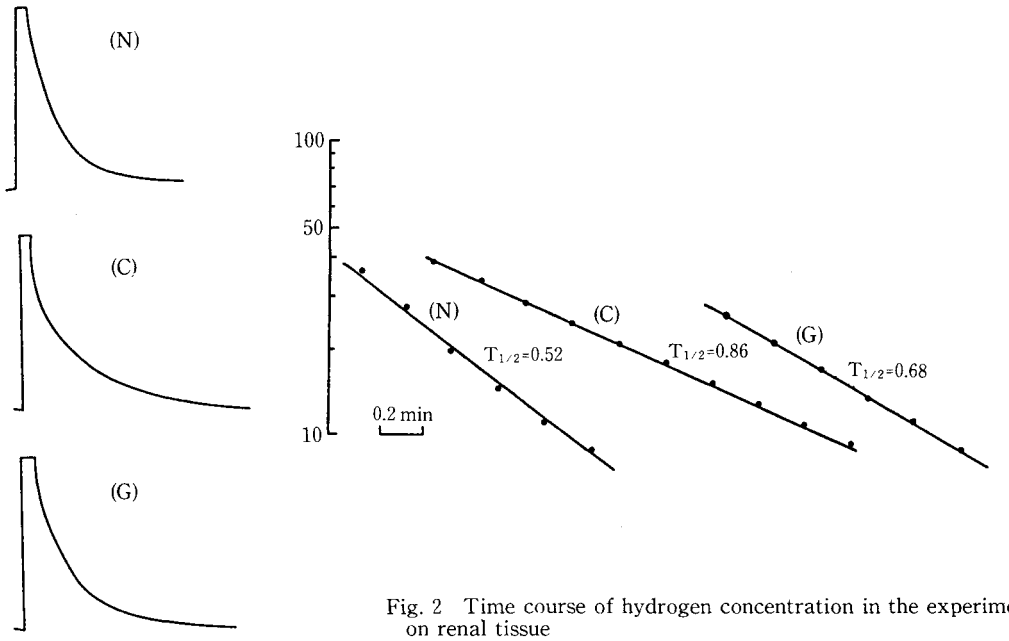

Fig. 2 Time course of hydrogen concentration in the experiment on renal tissue
(N), normal rat ; (C), chronic renal failure rat (control group) ; (G), chronic renal failure rat (red ginseng-treated group).

Discussion

Of substances derived from the urea cycle which seem to act as uremic toxins, the synthesis of MG is markedly increased in renal failure,[26] and its toxic effects including suppression platelet aggregation have been reported by Caharane *et al.*[27] MG has been extensively investigated with regard to its occurrence as a uremic toxin.[28] Like MG, GSA has also been found to be increased in the blood and urine of renal failure patients by Cohen *et al.*[29] and it has been shown to exhibit various toxic properties such as disturbance of platelet function, hemolytic activity, glucose metabolism, and inhibition of lymphocyte transformation in studies by Horowitz *et al.*,[30] Giovannetti *et al.*,[31] Cohen *et al.*,[32] and Slavin *et al.*[33] The fact that the ginseng we used reduced the accumulation in the body of guanidino compounds such as MG and GSA which are increased in the blood in renal failure might be suggestive of a certain beneficial effect, alleviating the uremic state.

On the other hand, in patients with renal failure, a decrease in renal blood flow and various morphological changes in the kidneys are observed ; because of fibrosis, blood vessels become compressed, tortuous and occluded, and thrombi and occlusion occur due to inflammation of the vessel walls or arteriosclerotic intimal tylosis. Decrease in the renal blood flow is accelerated with complications such as cardiac insufficiency, dehydration, electrolyte imbalance, *etc.*, in addition to the changes in the kidneys themselves, and renal blood directly influences the glomerular filtration rate.[34] In the measurements of blood flow using hydrogen gas generated by electrolysis, the adenine-fed rats showed a decrease in renal blood flow as compared with normal rats. Successive and simultaneous administration of adenine and ginseng produced a significant increase in renal tissue blood flow. It is conceivable that the increase of flow produced by ginseng could be attributable to the decrement in the accumulation of uremic toxins such as MG and GSA as described above. These effects of ginseng were in agreement with those which have been observed in rats given the traditional Chinese prescription "Onpi-tô (Wen-Pi-Tang)" composed of Rhei Rhizoma, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma, and Aconiti Japonici Tuber.[35]

However, the mode of action of ginseng is presumably different from that reported previously for Onpi-tô.[12,13] In rats given an adenine diet for 24 days, metabolic abnormalities have been shown to be produced such as hyperazotemia, accumulation of uremic toxins, metabolic imbalances of electrolytes and amino acids, and hypoalimentation accompanied by hypoalbuminemia, resembling those observed in human chronic renal failure.[16-19] The oral administration of Onpi-tô also causes marked reduction in serum urea nitrogen and creatinine levels, and improved hyperphosphatemia. As is obvious from the results of the present experiment, ginseng exhibited less of an effect on the levels of serum urea nitrogen, creatinine, and inorganic phosphate. From this it is thought that, unlike the ginseng-containing prescription Onpi-tô which produces improvement of metabolic abnormalities such as nitrogen and electrolyte imbalance, ginseng has a direct influence on the changes in the kidneys themselves, in view of the interesting finding that ginseng accelerates the renal blood flow rate. Further studies on the patho-histochemistry of renal tissue are currently in progress.

From ancient times, the roots of *Panax ginseng* C. A. MEYER have been used as a therapy for the wasting syndrome, rather than as a specific therapeutic agent, in various diseases including diabetes mellitus, atherosclerosis, and hypertension. Recently, the effect of a red ginseng prescription on symptoms of imbalance occurring during hemodialysis was examined clinically in 7 patients. Sato *et al.*[36] reported that ginseng as a form of uremic therapy suppressed subjective symptoms such as fatigue, palpitation, lumbago, dizziness, shoulder stiffness, anemia, and heavy breathing. Such observations show that ginseng has a certain clinically beneficial effect in the alleviation of the uremic state. The findings

obtained in the present experiment are thought to provide a partial explanation of the scientific criteria involved in the subjective symptoms.

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和文抄録

アデニン誘発慢性腎不全ラットに紅参を経口投与し，血中成分に対する効果を検討した。尿素窒素，クレアチニン，無機リン，カルシウム値に対しては低下が見られたが，メチルグアニジンレベルは7.5と15 mg/rat/day投与群において有意に低下した。またグアニジノコハク酸も15 mg/rat/day投与群において有意に低下した。さらに紅参処理ラットでは，腎組織血流量の増加も見られた。以上の実験成績をもとに，紅参による尿毒症状の改善についてdiscussionした。

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