Uremia-preventive effect of rhubarb extract in rats

Takako YOKOZAWA,*[a] Naomi SUZUKI,[a] Itsuko OKUDA,[a]
Hikokichi OURA[a] and Itsuo NISHIOKA[b]

[a] *Department of Applied Biochemistry, Research Institute for Wakan Yaku, Toyama Medical and Pharmaceutical University*
[b] *Faculty of Pharmaceutical Sciences, Kyushu University*

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Abstract

After renal failure was induced by administering adenine diet, rhubarb extract was administered to examine its effect on blood components. As a result, three days of adenine administration in a group prior to 18 days of administration of the extract led to marked reductions in urea nitrogen, guanidinosuccinic acid, and methylguanidine, suggesting improvement of uremic symptoms. Similar changes have been noted in the experimental group where 6 days of adenine diet were followed by 18 days administration of the extract. However, reduction of urea nitrogen and guanidino compounds by the extract was lowered in its intensity as the days of adenine prior to the extract administration was longer. In addition, there were no significant differences in the serum creatinine among three experimental groups.

Key words Rhei Rhizoma, chronic renal failure, uremia-preventive effect, urea nitrogen, guanidinosuccinic acid, methylguanidine, rat

Abbreviations GAA ; guanidinoacetic acid, GSA ; guanidinosuccinic acid, MG ; methylguanidine, TCA ; trichloroacetic acid

Introduction

We found that rats given adenine showed marked elevation of serum creatinine and urea nitrogen levels, increased methylguanidine and guanidinosuccinic acid, abnormalities in urea cycle and blood and urinary amino acid patterns, disturbed electrolyte metabolism, polyuria, hypobaric urine, *etc.* and that these changes intensified with an increase in the duration of administration periods.[1-8] We also found histologically that adenine induced marked renal failure, on the basis of observations of many polarization-positive needle crystals (2,8-dihydroxyadenine) in tubules and interstice obstructed tubules, granuloma formation, luminal and interstitial dilatation, epithelial degeneration, and further findings of tubular degeneration, necrosis, and exfoliation resulting in vacuolization.[3-5,8-10]

On the other hand, rats which were given rhubarb extract at the time of adenine administration tended to gain weight ; daily administration of rhubarb extract caused marked reduction in blood urea nitrogen and creatinine ; guanidinosuccinic acid and methylguanidine also decreased ; hypocalcemia, hyperphosphatemia, and free amino acid patterns improved ; suggesting improvement of uremic symptoms.[11,12]

In this paper, attempts were made to investi-

*〒 930-01 富山市杉谷 2630
富山医科薬科大学和漢薬研究所臨床利用部門 横澤隆子
2630, Sugitani, Toyama 930-01, Japan

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gate the effect of rhubarb extract in adenine-induced renal failure in terms of effect on blood urea nitrogen, creatinine, and guanidino compounds levels for its availability as a therapeutic agent.

Materials and Methods

Animals and treatments : Male Wistar strain rats of 5 weeks of age, initially weighing 110-120 g, were used in this experiment. The animals were fed *ad libitum* on 18 % casein diet containing 0.75 % adenine. The 18 % casein 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[13] 4 g, vitamin mixture[13] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added at the level of 0.75 g/100 g of the diet. This adenine feeding procedure produced experimental chronic renal failure.[1-10] Administration of adenine diet for 3 days (experiment 1), 6 days (experiment 2), or 12 days (experiment 3) was followed by 18 days of administration of the extract from Rhei Rhizoma (rhubarb extract-treated group), while control rats were given tap water. A dose of rhubarb extract was about 35 mg/rat/day during the experimental period. There was no statistically significant difference between the control and rhubarb extract-treated groups with regard to the body weight. Food intake of each group was almost proportional to the weight change throughout the experimental period. On the 6th, 12th, or 18th day of the administering period of the rhubarb extract, blood was collected by heart puncture under light ether anesthesia. They were allowed to clot at room temperature and were then centrifuged. The sera obtained were used for the determination of urea nitrogen. On the last day of the rhubarb extract treatment, rats were stunned by a sharp blow on the head and blood samples were collected in a conical centrifuge tube for the determination of creatinine and guanidino compounds.

Extraction of Rhei Rhizoma : Roots of *Rheum officinale* BAILLON produced in China were ground into a fine powder and extracted at 100℃ with water, as described previously.[14] The filtrate was concentrated under reduced pressure to obtain a brown residue.

Analyses : Urea nitrogen was determined by using a commercial reagent (Urea NB-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the urease-indophenol method.[15] Creatinine was determined by using a commercial reagent (Creatinine-Test Wako) based on the Folin-Wu method.[16] For the determination of guanidino compounds, serum was deproteinized by the addition of trichloroacetic acid (TCA) (final concentration, 10 %). The supernatant obtained by centrifugation at 3000 rpm for 10 min was applied to a Shimadzu LC-5A liquid chromatograph using a stepgradient. A fluorescence spectrometer, model RF-540 (excitation 395 nm, emission 500 nm ; Shimadzu Co.) was used to monitor the effluent from the column.

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

Results

Urea nitrogen : Three days of adenine administration prior to 18 days of administration of the rhubarb extract (experiment 1) led to marked

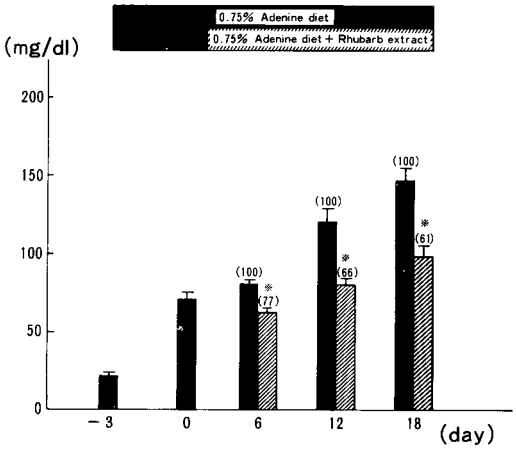

Fig. 1 Effect of rhubarb extract on urea nitrogen in the serum (experiment 1).
Values are means±S.E. of 10 rats. Figures in parentheses are percentages of the control value.
*Significantly different from the control value, $p<0.001$.

reduction in urea nitrogen. As shown in Fig. 1, the level of serum urea nitrogen was significantly lower at the 6th, 12th, and 18th days in rats of the rhubarb extract-treated group. In particular, the serum urea nitrogen level was decreased by 39 % at the 18th day as compared with the control group, indicating improvement of the uremia. In the next experiment, adenine diet was given to induce renal failure 6 days prior to the oral administration of rhubarb extract. The result of this experiment (experiment 2) is presented in Fig. 2. Rhubarb extract significantly reduced the urea nitrogen level by 16-25% at the 6th, 12th, and 18th days. On the other hand, when adenine diet was given for 12 days to induce a more severe state of renal failure (experiment 3), the reduction of urea nitrogen in the serum was no longer found (Fig. 3).

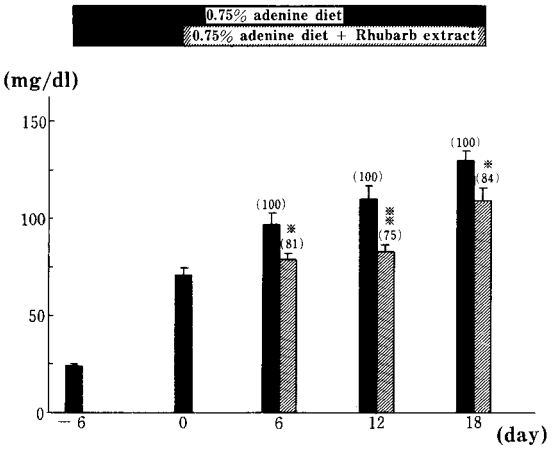

Fig. 2 Effect of rhubarb extract on urea nitrogen in the serum (experiment 2).
Values are means±S.E. of 10 rats. Figures in parentheses are percentages of the control value.
*Significantly different from the control value, $p<0.05$, $**p<0.01$.

Creatinine : Table I shows the creatinine level in the serum. There were no appreciable differences between the control and rhubarb extract-treated group throughout the three phases of the experiment.

Guanidino compounds : The concentrations of various guanidino compounds are shown in Table II. Administration of the rhubarb extract

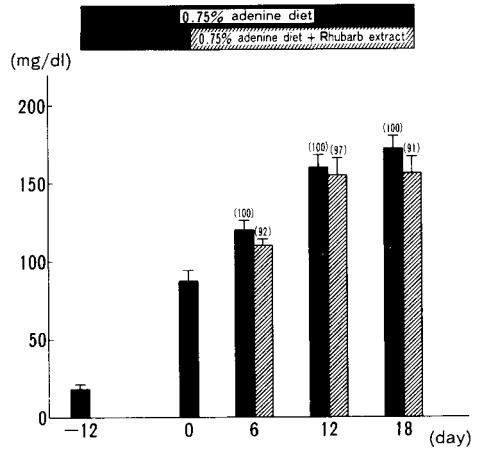

Fig. 3 Effect of rhubarb extract on urea nitrogen in the serum (experiment 3).
Values are means±S.E. of 10 rats. Figures in parentheses are percentages of the control value.

Table I Effect of rhubarb extract on creatinine level in the serum.

Exp. No.	Material	Creatinine (mg/dl)
1	Control	2.34±0.08 (100)
	Rhubarb extract	2.29±0.11 (98)
2	Control	2.33±0.18 (100)
	Rhubarb extract	2.18±0.11 (94)
3	Control	2.47±0.12 (100)
	Rhubarb extract	2.48±0.08 (100)

Values are means±S.E. of 10 rats. Figures in parentheses are percentages of the control value.

to rats resulted in a decrease of guanidino compounds. As shown in Table II, guanidinosuccinic acid (GSA) in the serum was 60 % lower after 3 days of adenine administration prior to 18 days of administration of the rhubarb extract (experiment 1) in comparison to the control group ($p<0.01$). The methylguanidine (MG) level in the serum of the rhubarb extract-treated group also decreased sharply. However, there were no differences between the control and rhubarb extract-treated groups in the level of serum

Table II Effect of rhubarb extract on levels of guanidino compounds in the serum.

Exp. No.	Material	GSA (μg/dl)	MG (μg/dl)	GAA (μg/dl)
1	Control	74.70±8.19 (100)	9.21±1.75 (100)	130.05±3.44 (100)
	Rhubarb extract	29.62±4.81** (40)	4.25±0.23* (46)	134.42±4.30 (103)
2	Control	93.47±4.10 (100)	8.51±0.64 (100)	150.76±16.01 (100)
	Rhubarb extract	71.89±3.93** (77)	5.44±0.35*** (64)	134.89±14.75 (89)
3	Control	67.33±3.34 (100)	14.71±1.07 (100)	71.64±6.44 (100)
	Rhubarb extract	57.65±4.55 (86)	9.86±0.96* (67)	89.90±5.14 (125)

GSA, guanidinosuccinic acid ; MG , methylguanidine ; GAA, guanidinoacetic acid. Values are means±S.E. of 10 rats. Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

guanidinoacetic acid (GAA). The reduction of GSA and MG levels was observed in experiment 2. Particularly, the level of MG was decreased to 5.44 μg/dl on the average. The value for serum MG was about 36 % lower in the rhubarb extract-treated group as compared with the control group. However, the rats given 12 days of adenine administration prior to 18 days of administration of the rhubarb extract showed no appreciable changes except in the case of MG.

Discussion

In renal failure, reduced excretion of nitrogen compounds is accompanied by elevated blood urea levels. The enterohepatic circulation of urea has found that urea is synthesized in the liver, excreted into the intestine partly, and decomposed there by urease of intestinal bacteria to ammonia, which is absorbed from the intestinal tract and turns to urea again in the liver.[17,18] Among conservative treatments of chronic renal failure, which make use of such enterohepatic circulation of urea, are the essential amino acid therapy[19] and the α-keto acid therapy.[20] These aim at improvement of hyperazotemia and correction of nitrogen balance through re-utilization of urea as a source for non-essential amino acid synthesis. To promote extra renal excretion of urea, administration of activated charcoal, ion-exchange regin, oxrstarch, methylcellulose, *etc.* have been attempted.[21-23]

Rhubarb extract which was used in the present study acts to promote the re-utilization system of urea and protein synthesis in the body of normal rats and to suppress muscular protein decomposition, for which a possible mechanism is a kind of anabolic and/or anti-catabolic action.[24] On the other hand, as reported previously,[11,12] successive and simultaneous administration of adenine and rhubarb extract caused marked reduction of serum urea nitrogen and creatinine levels, and liver urea contents were decreased nearly in parallel with serum urea nitrogen. Complete disappearance of serum MG, marked reduction of GSA, increased levels of Val, Ser, Thr, Leu, Ileu, Tyr, *etc.*, and improved hypocalcemia and hyperphosphatemia were observed. Rhubarb extract is considered to improve renal failure and prevent its progress by suppressing urea synthesis or improving disorders in the urea cycle and amino acid and electrolyte metabolism. In the present study, adenine was given to cause renal failure and then rhubarb extract was administered to investigate the effect of the extract.

As a result, it was found that 0.75 % adenine diet given to cause renal failure 3 or 6 days prior to the rhubarb extract administration resulted in

significant reduction in serum urea nitrogen in any groups as compared with the control group. In spite of lowered levels of serum urea nitrogen, creatinine levels were nearly constant with a consequent low ratio of urea nitrogen/creatinine. On the other hand, when adenine diet was given for 12 days to cause a more severe state of renal failure blood-biochemically and histologically, serum urea nitrogen reducing effects were no longer found. It has hitherto been reported that administration of essential amino acids under stringent protein restriction as a conservative therapy of renal failure promotes re-utilization of urea and corrects negative nitrogen balance.[19] Reduced serum urea nitrogen levels attained by rhubarb extract administration under 18% protein regimen may suggest an acceptability of some protein load and a possibility of improving the whole body condition.

Interesting findings were also obtained with regard to guanidino compounds : In groups where 3 days of adenine diet were followed by 18 days of administration of the extract, GSA and MG were markedly reduced. In groups where 6 days of adenine diet were followed by the extract, there was also a significant decrease in GSA and MG. These changes in guanidino compounds were greater than those in urea nitrogen, which was in agreement with the results obtained when the extract was given at the same time with adenine. On the contrary, there was no significant changes in other guanidino compounds other than MG in rats which were put on the extract when serum urea nitrogen was about 90 mg/dl after 12 days of adenine diet.

Of substances derived from the urea cycle which seem to be uremic toxins, MG was markedly increased in synthesis in renal failure,[25] and its toxic effects including platelet aggregation suppression have been reported by Caharane *et al.*[26] Investigation has been carried out extensively on it as one of uremic toxins.[27] Like MG, GSA has been found to increase in the blood and urine of renal failure patients by Cohen *et al.*[28] and it has been shown to exhibit various toxic activities such as platelet function disturbance, hemolytic activity, glucose metabolism disturbance, and inhibition of lymphocyte transformation by Horowitz *et al.*,[29] Giovanetti *et al.*,[30] Cohen *et al.*,[31] and Slavin *et al.*[32] Aoyagi *et al.*[33,34] have reported that in isolated hepatocyte experiments, urea stimulates GSA synthesis. Jones *et al.*,[35] Pfiffner *et al.*,[36] Perez *et al.*,[37] and Mikami *et al.*[38] have found that MG is synthesized from creatinine as a precursor. The fact that the extract we used reduced the accumulation in the body of nitrogen compounds such as urea nitrogen and guanidino compounds which are increased in the blood in renal failure may be suggestive of the usefulness of the extract as one of conservative therapies for chronic renal failure.

Based on the above findings, it is likely that rhubarb extract can delay progress of renal failure and improve various states of metabolic abnormality. Findings suggestive of metabolic improvements in renal failure have been obtained by us with rhubarb-containing prescriptions.[39] Furthermore, a recent case has been reported in which a patient with chronic renal failure responded well to medication of rhubarb or rhubarb-containing prescriptions.[40] Rhubarb has been accepted to have pharmacological properties such as purgative, antibacterial, astringent, stomachic, and cholagogic effects.[41] In a series of our studies, it was suggested that rhubarb has a possibility as a therapeutic agent for renal failure in view of its new pharmacological property, that is, improvement of body nitrogen metabolism and other factors.

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