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Effect of Orally Administered Rhubarb Extract in Rats with Chronic Renal Failure

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The effect of orally administered rhubarb extract was examined in rats with chronic renal failure induced by an adenine diet. On treatment of the rats with the rhubarb extract, the level of urea nitrogen and creatinine in the serum was dose-dependently decreased. The urea concentration in the liver was also decreased after the treatment. In addition, administration of the rhubarb extract to rats produced an increase in the serum calcium level, indicating an improvement of hypocalcemia. Improvement of hyperphosphatemia was also observed. Furthermore, rhubarb extract appeared to cause a gradual decrease of the taurocyamine, guanidinosuccinic acid, and methylguanidine levels in the serum with increasing dosage. Methylguanidine was not detectable in the serum or in the kidney of the rhubarb extract-treated group given 55 mg/rat/d. A marked decrease in the liver guanidinosuccinic acid concentration was also observed. Treatment of chronically uremic rats with the rhubarb extract resulted in a normal or nearly normal serum level of branched-chain amino acids. These results suggest that rhubarb extract may be useful as a conservative treatment for uremia.

Keywords——rhei rhizoma; renal failure; blood urea nitrogen; serum creatinine; liver urea; serum calcium; serum phosphate; guanidino compound; free amino acid; rat

Our previous report showed that the extract from Rhei Rhizoma caused a significant decrease in the levels of both urea nitrogen and creatinine in the serum after repeated intraperitoneal administration in rats with chronic renal failure induced by an adenine diet. In particular, the creatinine level was decreased by 12—18% at the 6—24th days as compared with the control value, indicating an improvement of renal function.[1] Moreover, administration of the rhubarb extract to rats caused a significant increase in the urinary excretion of both urea and creatinine.[2] These results indicate that rhubarb extract improves the renal clearance in the uremic state and suggest that the progression of renal failure may be stopped. These experimental findings also raise the possibility that rhubarb extract treatment may be a useful therapy for uremia. To evaluate the possible therapeutic significance of rhubarb extract, oral administration of rhubarb extract to rats with chronic renal failure was carried out.

Materials and Methods

Animals and Treatments——Male rats of the JCL: Wistar strain, initially weighing 110—120 g, were used in this experiment. The animals were fed *ad libitum* on 18% casein diet containing 0.75% adenine for 24 d. 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[3] 4 g, vitamin mixture[3] 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 diet. This procedure of adenine feeding produced experimental chronic renal failure.[4–10] During the adenine feeding period, the extract from Rhei Rhizoma was administered orally to rats (rhubarb extract-

treated group), while control rats were treated with an equal volume of tap water.

Extraction of Rhei Rhizoma——Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted at 100 °C with water, as described previously.[11] The filtrate was concentrated under reduced pressure to obtain a brown residue.

Analyses——On the 24th day of the feeding period, rats were sacrificed by means of a blow on the head and exsanguinated. Blood was collected in a conical centrifuge tube for the determination of urea nitrogen, creatinine, calcium, phosphate, guanidine compounds, and free amino acids. 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.[12] Creatinine was determined by using a commercial reagent (Creatinine-Test Wako) based on the Folin-Wu method.[13] Calcium was determined by using a commercial reagent (Calcium C-Test Wako) based on the orthocresol-phthalein complex compound method.[14] Inorganic phosphate was determined by using a commercial reagent (Phosphor B-Test Wako) based on the molybdenum blue method.[15] 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. Free amino acids were determined with a Hitachi 835 high-speed amino acid analyzer. Before this determination, the serum was deproteinized by adding 3 volumes of 3% sulfosalicyclic acid.

Liver and kidneys were removed quickly, cooled on ice, and weighed rapidly. A portion of the liver was homogenized with 9 volumes of ice-cold water in a Potter-Elvehjem type glass homogenizer. The homogenate, diluted about 100-fold with water, was used for the determination of urea by the method of Archibald.[16] For the determination of guanidino compounds, portions of the liver and kidney were deproteinized with TCA and the supernatant obtained by centrifugation at 3000 rpm for 10 min was applied to a Shimadzu LC-5A liquid chromatograph.

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

Results

Effect of Rhubarb Extract on Urea Nitrogen and Creatinine Levels in the Serum

To compare the biological activities of the extract from Rhei Rhizoma, the dose–response relationship was determined. Table I shows the effect of the rhubarb extract on urea nitrogen and creatinine levels in the sera of rats of the rhubarb extract-treated and control groups. Rhubarb extract significantly reduced the urea nitrogen level by 19—44% at 15—55 mg/rat/d as compared with the control, while the oral administration of 5 mg/rat/d showed no effect. The creatinine level was also decreased by 16—41% as compared with the control upon oral administration of 5—55 mg/rat/d. The effect of rhubarb extract on urea nitrogen and creatinine levels was dose-dependent.

Effect of Rhubarb Extract on Urea Concentration in the Liver

The liver is known to be the site of ureapoiesis, since it contains all the enzymes of the urea cycle, and urea synthesis takes place in this organ.[17,18] Therefore, it seemed interesting to

TABLE I. Effect of Rhubarb Extract on Urea Nitrogen and Creatinine Levels in the Serum

Material	Dose (mg/rat/d)	Urea nitrogen (mg/100 ml)	Creatinine (mg/100 ml)
Control	—	91.2±5.0 (100)	3.00±0.18 (100)
Rhubarb extract	5	83.8±5.8 (92)	2.50±0.07[a] (84)
Rhubarb extract	15	73.7±4.0[a] (81)	2.18±0.07[a] (73)
Rhubarb extract	35	72.3±4.1[a] (79)	2.15±0.15[b] (72)
Rhubarb extract	55	50.8±6.7[c] (56)	1.78±0.10[c] (59)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

Table II. Effect of Rhubarb Extract on Urea Concentration in the Liver

Material	Dose (mg/rat/d)	Liver urea (mg/g tissue)
Control	—	1.45±0.06 (100)
Rhubarb extract	5	1.29±0.09 (89)
Rhubarb extract	15	1.18±0.05[a] (81)
Rhubarb extract	35	1.17±0.05[a] (81)
Rhubarb extract	55	0.91±0.08[b] (63)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.01$, *b*) $p<0.001$.

Table III. Effect of Rhubarb Extract on Calcium and Phosphate Levels in the Serum

Material	Dose (mg/rat/d)	Ca (mg/100 ml)	P (mg/100 ml)
Control	—	5.89±0.18 (100)	16.21±0.33 (100)
Rhubarb extract	5	6.26±0.14 (106)	14.55±0.60[a] (90)
Rhubarb extract	15	6.93±0.30[a] (118)	13.98±0.84[a] (86)
Rhubarb extract	35	7.00±0.25[b] (119)	13.95±0.83[a] (86)
Rhubarb extract	55	7.17±0.24[b] (122)	13.13±0.99[a] (81)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$.

determine whether or not the oral administration of rhubarb extract modified the urea concentration in the liver. The result of this experiment is presented in Table II. On treatment of rats with the rhubarb extract (15—55 mg/rat/d), the urea concentration in the liver was significantly decreased by 19—37% as compared with the control. The reduction of urea was proportional to that of urea nitrogen level in the serum.

Effect of Rhubarb Extract on Calcium and Phosphate Levels in the Serum

The results of our previous work have so far shown that the administration of adenine to rats significantly decreased the level of serum calcium, while the serum phosphate level showed a significant increase.[10] Oral administration of the extract from Rhei Rhizoma to rats fed on the adenine diet caused a significant increase of the calcium level in the serum. As shown in Table III, the value for serum calcium was about 22% higher at the dosage level of 55 mg/rat/d in the rhubarb extract-treated group as compared with the control group. In addition, the level of serum phosphate depended on the amount of rhubarb extract administered to rats; the administration of 55 mg/rat/d of the rhubarb extract decreased it by 19% of the control value.

Effect of Rhubarb Extract on Guanidino Compounds in the Serum, Liver, and Kidney

The concentrations of various guanidino compounds are shown in Table IV. Among the various guanidino compounds present in the serum, the control group contained six in significant amounts, namely taurocyamine (TAU), guanidinosuccinic acid (GSA), guanidinoacetic acid (GAA), guanidinopropionic acid (GPA), guanidinobutyric acid (GBA), and methylguanidine (MG). Administration of the rhubarb extract to rats resulted in a decrease of guanidino compounds in the serum. As shown in Table IV, TAU in the serum was about 15—29% lower at the dosage level of 15—55 mg/rat/d as compared with the control group

TABLE IV. Effect of Rhubarb Extract on Levels of Guanidino Compounds in the Serum

Material	Dose (mg/rat/d)	TAU (μg/100 ml)	GSA (μg/100 ml)	GAA (μg/100 ml)	GPA (μg/100 ml)	GBA (μg/100 ml)	MG (μg/100 ml)
Control	—	124.9±3.5 (100)	69.5± 7.0 (100)	99.0±4.3 (100)	12.0±1.8 (100)	24.2±2.4 (100)	11.1±1.1 (100)
Rhubarb extract	5	124.3±5.9 (100)	62.7±13.4 (90)	88.0±8.8 (89)	17.4±4.1 (145)	22.5±2.4 (93)	7.1±0.5[a] (64)
Rhubarb extract	15	106.1±5.2[a] (85)	60.6± 7.6 (87)	96.8±6.2 (98)	—	18.4±4.4 (76)	—
Rhubarb extract	35	109.4±3.4[a] (88)	38.8± 3.8[b] (56)	72.4±3.3[b] (73)	12.6±1.2 (105)	16.1±1.6[a] (67)	6.2±0.5[c] (56)
Rhubarb extract	55	88.4±3.6[c] (71)	28.0± 1.1[c] (40)	90.1±4.9 (91)	11.5±1.6 (96)	20.9±0.8 (86)	N.D.

TAU, taurocyamine; GSA, guanidinosuccinic acid; GAA, guanidinoacetic acid; GPA, guanidinopropionic acid; GBA, guanidinobutyric acid; MG, methylguanidine.

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value. *a*) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. N.D., not detectable.

TABLE V. Effect of Rhubarb Extract on Levels of Guanidino Compounds in the Liver and Kidney

	Material	Dose (mg/rat/d)	GSA (μg/tissue)	GAA (μg/tissue)	MG (μg/tissue)
Liver	Control	—	28.9±3.6 (100)	28.7± 4.1 (100)	N.D.
	Rhubarb extract	55	14.2±2.5[a] (49)	36.1± 6.0 (126)	N.D.
Kidney	Control	—	9.7±1.0 (100)	67.0± 7.4 (100)	4.0±0.5 (100)
	Rhubarb extract	55	5.5±2.2 (57)	90.7±11.6 (135)	N.D.

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.01$. N.D., not detectable.

($p<0.001$ at 55 mg/rat/d). The GSA level in the serum of the rhubarb extract-treated group was sharply decreased at the 35 and 55 mg/rat/d levels. However, there was no difference between the control and rhubarb extract-treated groups at the dosage levels of 5 and 15 mg/rat/d. Furthermore, MG, which plays a part in uremic toxicity, was about 36% lower in the 5 mg/rat/d group as compared with the control group. The level of MG was decreased to 6.2 mg/100 ml on average at 35 mg/rat/d and was not detectable at 55 mg/rat/d. A decrease was also observed in the level of serum GAA and GBA at the 35 mg/rat/d dose level.

Table V shows tissue concentration of guanidino compounds. Generally, GSA concentration in the liver was higher than in the kidneys, but GAA concentration in the kidneys was higher than in the liver. MG was not detectable in the liver. As shown in Table V, the administration of 55 mg/rat/d of the rhubarb extract caused a significant decrease of about 50% in GSA concentration in the liver. In addition, MG was not detectable in the kidneys in the rhubarb extract-treated group. However, there was no statistically significant difference between the control and rhubarb extract-treated groups with regard to the GAA concentration in the kidneys.

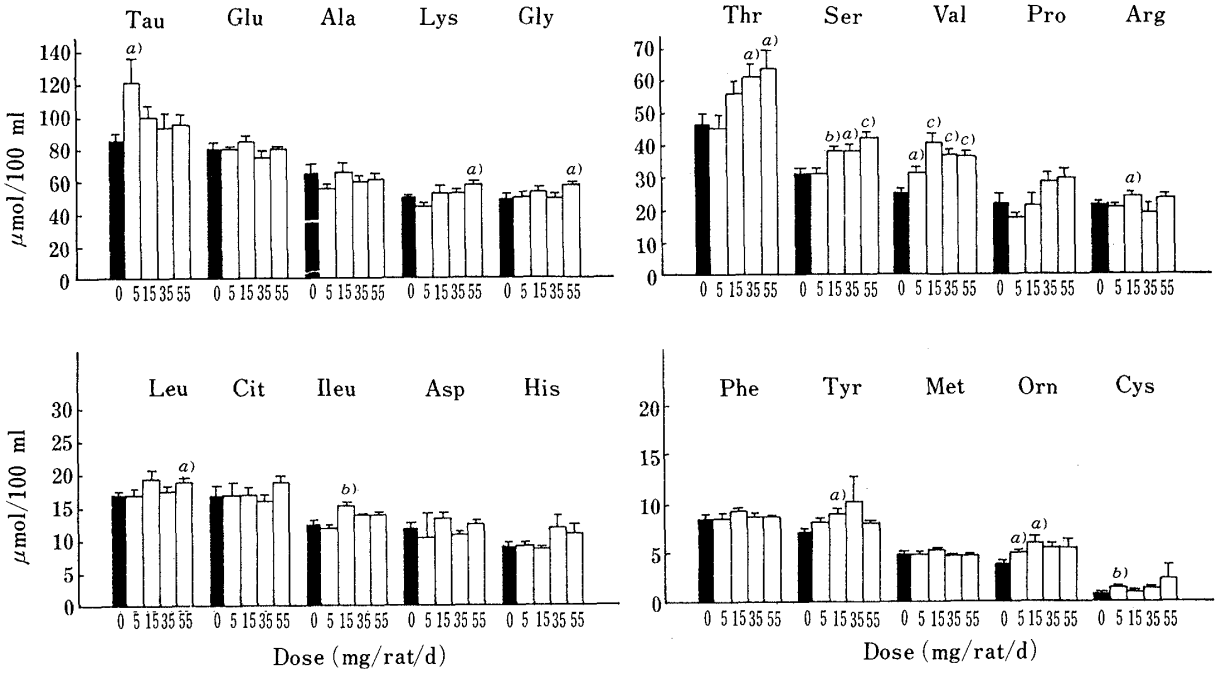

Fig. 1. Effect of Rhubarb Extract on Free Amino Acids in the Serum

Values are means ± S.E. of 6 rats. *a*) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

Effect of Rhubarb Extract on Free Amino Acids in the Serum

The levels of free amino acids in the serum are shown in Fig. 1. Though the data are not shown, adenine-fed rats showed a number of significant differences as compared with rats given the 18% casein diet. In particular, serum tyrosine was significantly lower in the adenine group. Lysine, valine, leucine, isoleucine, and ornithine were also significantly reduced in the adenine-fed rats, whereas glycine, arginine, and aspartic acid were significantly increased. The levels of essential amino acids were significantly reduced in the serum from the adenine-fed rats.[9] In an examination of the effects of oral administration of the extract from Rhei Rhizoma, it was found that the level of amino acids in the serum was significantly increased. As shown in Fig. 1, rhubarb extract induced an increase of serum threonine and serine levels in a dose-dependent manner at doses of 5 to 55 mg/rat/d. Valine level, which was 31.7 μmol/100 ml at a dose of 5 mg/rat/d, was increased to 40.6 μmol/100 ml at a dose of 15 mg/rat/d. The ornithine level was also increased to 6.1 μmol/100 ml on average at a dose of 15 mg/rat/d. However, further increase in the dose to 35 and 55 mg/rat/d did not produce any further increase in valine or ornithine level.

Discussion

In this work we present evidence that the extract from Rhei Rhizoma produces an improvement of renal function in the uremic state induced by adenine feeding, as demonstrated by significant decreases in serum urea nitrogen and creatinine, correction of hypocalcemia and hyperphosphatemia, disappearance of methylguanidine in the serum and kidney, and improvement in the serum amino acid concentration pattern.

As shown in Table I, the level of serum urea nitrogen was significantly lower at doses of 15, 35, and 55 mg/rat/d in rats of the rhubarb extract-treated group. In addition, serum creatinine level was decreased by 41% at a dose of 55 mg/rat/d as compared with the control group. The urea concentration in the liver was also decreased significantly upon oral

administration of 15, 35, and 55 mg/rat/d of rhubarb extract (Table II). These results are better than those of repeated intraperitoneal administration reported previously.[1] The improvement of the serum concentrations of urea nitrogen and creatinine might be related to the serum level or the metabolic production of MG, GSA, and so forth.

Uremia is likely to produce excess urea and other nitrogenous fragments, probably including MG and GSA. MG and GSA are known to be highly concentrated in the serum of uremic patients.[19-22] Recently, MG and GSA have been found to have many toxic effects *in vitro*, and MG brings about the same symptoms as those of uremia in normal dogs subjected to repeated injection to raise the serum level to that found in the uremic state.[19,23] These experimental findings suggest that MG and GSA play an important role in the uremic state. In the previous paper, we demonstrated the presence of GSA and MG in the serum of rats with chronic renal failure induced by an adenine diet, and were unable to detect it in normal rats. On the other hand, uremic rats showed a significant decrease in serum GAA.[9] Our observations in the present study further showed the existence of TAU, GBA, and GPA in the serum of uremic rats (Table IV). Because the six guanidino compounds were found in rats fed on an adenine diet, the influence of rhubarb extract on the levels of these guanidino compounds in the serum was investigated.

As shown in Table IV, rhubarb extract decreased the MG and GSA levels in a dose-dependent manner. The MG level of the rhubarb extract-treated group at a dose of 55 mg/rat/d was not detectable. Another important finding is that the TAU level was decreased significantly at a dose of over 15 mg/rat/d, though the GAA, GPA, and GBA levels showed no clear dose-related decrease. After treatment of rats with the rhubarb extract, MG was not detectable in the kidney, and a remarkable decrease in the liver GSA concentration was observed (Table V). These results imply that not only the serum concentration but also the metabolic production of urea and other nitrogenous fragments (probably including MG and GSA) is decreased, corresponding to a positive nitrogen balance.

In addition, the changes in the guanidino compounds, especially MG and GSA, were found to correlate significantly with the serum urea nitrogen and creatinine level (Tables IV and I). Cohen reported that GSA was produced by transamidination from arginine to aspartate in the liver. The increase of GSA production in uremia may be caused by the diversion of arginine no longer used in the production of GAA.[24] However, Mikami *et al.* proposed that the increase of GSA production in uremia is closely related to the retention of urea rather than the retention of arginine by repression of arginine–glycine amidinotransferase in the liver.[25] Additionally, they demonstrated the metabolic origin of MG, *i.e.*, MG may be produced from creatinine.[25] Our observations in the present study are also compatible with the hypothesis of Mikami and his co-workers that most MG is produced through creatinine and that GSA production may be closely related to urea.

Another important observation was that the administration of the rhubarb extract to adenine-fed rats produced an increase in the serum calcium level, indicating an improvement of hypocalcemia. Improvement of the hyperphosphatemia was also observed (Table III). By far the most common cause of hyperphosphatemia is a decrease in urinary phosphate excretion secondary to renal failure. However, hyperphosphatemia can be the consequence of an increased entrance of phosphate into the extracellular fluid arising from tissue breakdown.[26] In addition, it is generally accepted that serum phosphate level may affect the metabolism of vitamin D; vitamin D is first metabolized by the liver into 25-hydroxycholecalciferol and then by the kidney to its active metabolite, 1,25-dihydroxycholecalciferol. Hyperphosphatemia may produce a fall in ionized calcium not only by precipitation of this ion but also by impairing the absorption of calcium from the intestine by decreasing the formation of 1,25-dihydroxycholecalciferol. Skeletal resistance to the action of parathyroid hormone (PTH) may also be a consequence of the decreased production of 1,25-dihydroxy-

cholecalciferol.[26] Considering these observations, further studies seem desirable to elucidate the nature of the improvement of renal function in rats with chronic renal failure induced by adenine.

On the other hand, the amino acid level is also considered to be a key index of the disease condition in chronic renal failure. In a recent compilation of the blood concentrations of amino acids in chronically uremic patients, it was pointed out that the concentrations of valine, isoleucine, and leucine were found to be subnormal in almost all reports.[27] Moreover, several specific defects in amino acid metabolism have been identified in renal failure, including impaired conversion of citrulline to arginine,[28] impaired hydroxylation of phenylalanine,[29-31] accelerated destruction of valine,[32] and altered protein-binding of tryptophan.[33] As reported previously, various differences were observed independently of the experimental period in our experimental animal model of chronic renal failure, *e.g.*, a decreased level of lysine, valine, leucine, tyrosine, isoleucine, and ornithine.[9] On the other hand, glycine, arginine, and aspartic acid were significantly elevated.[9] However, administration of the rhubarb extract to these rats was found to reduce or reverse the amino acid abnormalities of chronic renal failure. As shown in Fig. 1, treatment of chronically uremic rats with the rhubarb extract results in normal or nearly normal serum levels of branched-chain amino acids. In particular, the valine level rose significantly at all dosages. In mammals, valine, leucine, and isoleucine are metabolized primarily in the muscle, whereas most other amino acids are metabolized mainly in the liver.[34] Hence, a high branched-chain amino acid level may indicate altered metabolism in the muscle. Moreover, threonine and serine showed a progressive increase to the normal level at a dose of 55 mg/rat/d. Though the mechanism of these effects remains to be elucidated, the rhubarb extract treatment was clearly effective for improvement of the serum amino acid concentration pattern.

For uremic subjects, it is especially important to ensure that the total intake of amino acids is sufficient to maintain nitrogen equilibrium, but is not excessive; excessive nitrogen intake would lead to unnecessary accumulation of waste nitrogen. Several studies have documented the efficacy of supplements containing nitrogen-free analogues of the branched-chain amino acids, phenylalanine, and methionine plus the remaining essential amino acids when added to a diet adequate in calories and containing protein restricted as to quality.[35,36] Our observations in the present study suggest the usefulness of rhubarb extract, which is an important component of crude drug prescriptions in kanpo medicine, in the conservative treatment of uremia.

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