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Changes in the Urinary Constituents in Rats with Chronic Renal Failure during Oral Administration of Rhubarb Extract

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The effect of rhubarb extract on the urinary constituents was examined in rats with chronic renal failure induced by adenine. Orally administered rhubarb extract caused a significant decrease in the urinary excretion of urea. The amount of urinary Na, K, and Ca was significantly reduced in the rhubarb extract-treated rats, while urinary inorganic phosphate was significantly elevated. A polyuria-preventive effect was observed. However, there was no marked change in the amount of urinary creatinine throughout the experimental period. The mechanism of uremia-improving action of rhubarb extract is discussed on the basis of the present results and the previously reported data [*Chem. Pharm. Bull.*, **32**, 4506 (1984)].

Keywords——Rhei Rhizoma; chronic renal failure; urinary urea; urinary creatinine; urinary electrolyte; polyuria-preventive effect

In the previous work we presented 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.[1] These experimental findings raise the possibility that rhubarb extract treatment may be a useful therapy for uremia. However, there was insufficient information available on the usefulness of rhubarb extract in the conservative treatment of uremia. The present paper therefore deals with the effect of the extract on the urinary constituents in rats.

Materials and Methods

Animals and Treatment——Male rats of the JCL: Wistar strain, initially weighing 100—110 g, were placed in a metabolic chamber under a conventional lighting regimen with a dark-night period. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, type CE-2) for one week after arrival. Then they were fed *ad libitum* on an 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[2] 4 g, vitamin mixture[2] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. The procedure of adenine feeding produced experimental chronic renal failure.[3-12] During the adenine feeding period, the extract from Rhei Rhizoma was administered orally to rats as drinking water (rhubarb extract-treated group), while control rats received tap water (control group). The dose of rhubarb extract was about 35 mg/rat/d during the experimental period. The 48-h urine samples were each collected in a 100 ml Erlenmeyer flask. 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 essentially proportional to the weight change throughout the experimental period.

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.[13] The filtrate was concentrated under reduced pressure to

obtain a brown residue.

Analytical Methods——All reagents were commercial products of the highest grade available. a) Urea was determined by the method of Archibald.[14] b) Creatinine was determined by using a commercial reagent ("Creatinine-Test Wako" obtained from Wako Pure Chemical Industries) based on the Folin–Wu method.[15] c) Sodium and potassium were determined with an electrolyte measurement apparatus (AHS/Japan Corporation) based on the ion electrode method. d) Calcium was determined by using a commercial reagent ("Calcium C-Test Wako" obtained from Wako Pure Chemical Industries) based on the orthocresol–phthalein complex compound method.[16] e) Inorganic phosphate was determined by using a commercial reagent ("Phosphor B-Test Wako" obtained from Wako Pure Chemical Industries) based on the molybdenum blue method.[17]

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

Results

Urine Volume

Figure 1 compares the urine volume of the rhubarb extract-treated and control rats. The feeding of adenine diet (control group) produced a significant increase to 38.2—71.4 ml/2 d, while the urine volume in rats fed on a 18% casein diet was about 28.0 ml/2 d during the feeding period (data not shown). Adenine administered orally caused polyuria. Under these dietary conditions, the urine volume of the rhubarb extract-treated group was decreased by about 20% from that of the control group. In particular, the urine volume was significantly lower at days 0—2, 6—8, 12—14, 18—20, 20—22, and 22—24 in rats of the rhubarb extract-treated group. The reduction of urine volume was calculated to be 30% as compared with the control group throughout the experimental period. On the other hand, there were no appreciable changes in the water intake of the control and rhubarb extract-treated groups.

Urea

Table I compares the amount of urinary urea of the rhubarb extract-treated and control rats. In rats given the adenine diet (control group), reduced excretion of nitrogen compounds is accompanied by elevated blood urea, creatinine, guanidino compounds, and so forth.[5,11] Oral administration of the extract from Rhei Rhizoma to rats caused a marked decrease of the urea output in the urine; the greatest decrease was by about 30—50% at days 12—18.

Creatinine

As shown in Table I, there were no significant changes in the amount of urinary creatinine in rats of the rhubarb extract-treated and control groups.

Electrolytes

Adenine-fed rats exhibited a significant increase in the amounts of urinary Na, K, and Ca, while the urinary excretion of inorganic phosphate showed a significant decrease during the feeding period as compared with the control group.[10] Table II compares the electrolyte levels in the urine of the rhubarb extract-treated and control rats on an adenine diet. The

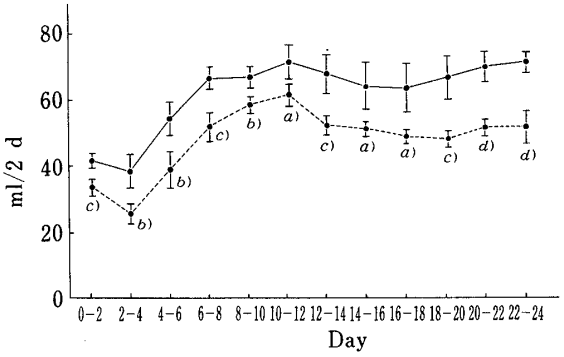

Fig. 1. Effect of Rhubarb Extract on Urine Volume

●—●, control group; ●---●, rhubarb extract-treated group. Values are means ± S.E. of 5 to 6 rats. Significantly different from the control group, *a*) $p<0.20$, *b*) $p<0.10$, *c*) $p<0.05$, *d*) $p<0.01$.

TABLE I. Effect of Rhubarb Extract on Urea and Creatinine Levels in the Urine of Rats

	Day	Control	Rhubarb extract
Urea (mg/2 d)	0— 2	479.8±18.6 (100)	496.2±45.0 (103)
	2— 4	662.4±67.4 (100)	469.0±33.0[b] (71)
	4— 6	643.2±79.0 (100)	613.4±55.0 (91)
	6— 8	466.6±66.2 (100)	579.6±77.6 (124)
	8—10	580.6±20.4 (100)	457.2±44.2[b] (79)
	10—12	585.6±44.0 (100)	543.4±27.2 (93)
	12—14	638.8±96.4 (100)	431.2±48.2[a] (68)
	14—16	773.8±25.8 (100)	403.4±26.4[c] (52)
	16—18	560.2±74.8 (100)	378.0±94.4[a] (67)
	18—20	480.2±77.0 (100)	353.0±32.8[a] (74)
	20—22	624.8±48.0 (100)	583.8±45.0 (93)
	22—24	626.0±76.8 (100)	565.0±98.4 (90)
Creatinine (mg/2 d)	0— 2	8.40±0.28 (100)	8.06±0.44 (96)
	2— 4	7.24±0.74 (100)	5.76±1.12 (80)
	4— 6	6.90±0.34 (100)	6.94±0.62 (101)
	6— 8	5.96±0.74 (100)	5.64±1.36 (95)
	8—10	7.58±0.20 (100)	6.86±0.82 (91)
	10—12	6.88±0.76 (100)	6.50±0.74 (94)
	12—14	6.86±0.40 (100)	7.70±0.48 (112)
	14—16	6.02±0.74 (100)	5.00±0.96 (83)
	16—18	5.84±0.48 (100)	6.70±0.74 (115)
	18—20	4.40±0.68 (100)	4.32±0.48 (98)
	20—22	4.76±0.88 (100)	5.32±1.02 (112)
	22—24	4.50±0.72 (100)	4.28±1.02 (95)

Values are means±S.E. of 5 to 6 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, *a*) $p<0.20$, *b*) $p<0.05$, *c*) $p<0.001$.

value for urinary Na was about 17—31% lower at days 16—18, 20—22, and 22—24 in the rhubarb extract-treated group as compared with the control group. The urinary excretion of K in the rhubarb extract-treated group was also decreased by 12—28% at days 16—18, 20—22, and 22—24. However, the urinary output of Na and K at days 10—12 was significantly elevated. Furthermore, the level of Ca showed a significant decrease, being about 41—54% lower at days 8—10, 12—14, 14—16, 18—20, and 20—22. On the other hand, rhubarb extract significantly increased the level of urinary inorganic phosphate from 21.2 to 27.8 mg at days 4—6. The increment of inorganic phosphate level was calculated to be 31% as compared with the control group. The level of urinary inorganic phosphate at days 10—12 was also increased. However, a decrease (14—15% compared to the control) was observed in the urine of the rhubarb extract-treated group at days 20—22 and 22—24.

Discussion

In chronic renal failure, azotemia is recognized and its severity can be estimated in terms of the extent to which the serum creatinine and/or the blood urea nitrogen (BUN) concentration are elevated above normal. The evaluation of changes in the concentration of serum creatinine or BUN requires an understanding of some basic principles of physiology and metabolism.[18]

For uremic subjects, it is generally important to ensure that the total intake of amino acids is sufficient to maintain nitrogen equilibrium, but is not excessive; excessive nitrogen

TABLE II. Effect of Rhubarb Extract on Electrolyte Levels in the Urine of Rats

	Day	Control	Rhubarb extract
Na (mM/2d)	0— 2	2.02±0.10 (100)	1.84±0.14 (91)
	2— 4	0.88±0.20 (100)	0.84±0.20 (95)
	4— 6	1.54±0.26 (100)	1.36±0.36 (88)
	6— 8	2.28±0.22 (100)	1.86±0.28 (82)
	8—10	1.96±0.22 (100)	2.18±0.18 (111)
	10—12	1.92±0.06 (100)	2.26±0.16[b] (118)
	12—14	1.52±0.18 (100)	1.72±0.18 (113)
	14—16	1.36±0.12 (100)	1.44±0.12 (106)
	16—18	1.66±0.16 (100)	1.38±0.10[a] (83)
	18—20	1.56±0.12 (100)	1.36±0.16 (87)
	20—22	2.46±0.08 (100)	1.82±0.08[e] (74)
	22—24	2.42±0.08 (100)	1.66±0.14[d] (69)
K (mM/2d)	0— 2	2.10±0.10 (100)	2.18±0.14 (104)
	2— 4	1.22±0.20 (100)	1.10±0.08 (90)
	4— 6	1.48±0.10 (100)	1.36±0.16 (92)
	6— 8	1.46±0.12 (100)	1.50±0.12 (103)
	8—10	1.52±0.14 (100)	1.60±0.06 (105)
	10—12	1.50±0.06 (100)	1.72±0.08[b] (115)
	12—14	1.48±0.10 (100)	1.36±0.06 (92)
	14—16	1.36±0.20 (100)	1.26±0.04 (93)
	16—18	1.32±0.04 (100)	1.16±0.06[b] (88)
	18—20	1.20±0.04 (100)	1.16±0.06 (97)
	20—22	1.54±0.06 (100)	1.34±0.06[b] (87)
	22—24	1.64±0.12 (100)	1.18±0.12[c] (72)
Ca (mg/2d)	0— 2	0.50±0.06 (100)	0.52±0.14 (104)
	2— 4	0.12±0.02 (100)	0.14±0.02 (117)
	4— 6	0.40±0.08 (100)	0.36±0.10 (90)
	6— 8	0.36±0.10 (100)	0.28±0.12 (78)
	8—10	0.46±0.04 (100)	0.26±0.08[b] (57)
	10—12	0.46±0.04 (100)	0.38±0.14 (83)
	12—14	0.34±0.10 (100)	0.20±0.02[a] (59)
	14—16	0.40±0.06 (100)	0.20±0.04[c] (50)
	16—18	0.36±0.06 (100)	0.36±0.06 (100)
	18—20	0.38±0.10 (100)	0.22±0.06[a] (58)
	20—22	0.48±0.12 (100)	0.22±0.08[b] (46)
	22—24	0.60±0.04 (100)	0.50±0.06 (83)
P (mg/2d)	0— 2	35.4±2.0 (100)	32.8±2.0 (93)
	2— 4	35.6±0.6 (100)	33.2±2.2 (93)
	4— 6	21.2±0.8 (100)	27.8±2.2[c] (131)
	6— 8	24.0±1.6 (100)	23.6±1.8 (98)
	8—10	23.4±0.6 (100)	25.6±1.6 (109)
	10—12	23.0±1.4 (100)	26.0±0.8[b] (113)
	12—14	26.6±2.6 (100)	25.4±0.2 (95)
	14—16	25.2±1.4 (100)	23.6±2.0 (94)
	16—18	19.0±2.0 (100)	21.6±1.2 (114)
	18—20	17.4±2.0 (100)	17.0±1.4 (98)
	20—22	23.4±1.6 (100)	20.0±1.6[a] (85)
	22—24	25.4±1.4 (100)	21.8±1.6[a] (86)

Values are means±S.E. of 5 to 6 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, *a*) $p<0.20$, *b*) $p<0.10$, *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$.

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.[19,20]

Our observations in the preceding study suggested the usefulness of rhubarb extract, which is an important component of crude drug prescriptions in so-called kanpo medicine, in the conservative treatment of uremia.[1] That is, treatment of chronically uremic rats with rhubarb extract produced an improvement of renal function in the uremic state induced by adenine feeding, as demonstrated by significant decreases in serum urea nitrogen and creatinine. The urea concentration in the liver, which is known to be the site of ureapoiesis,[21,22] was also decreased significantly upon oral administration of rhubarb extract. In the present experiment, the effect on the urinary constituents was further studied in order to evaluate the possible therapeutic significance of the rhubarb extract.

As shown in Table I, treatment of chronically uremic rats with the rhubarb extract resulted in a significant decrease in the urinary excretion of urea at days 2—4, 8—10, and 12—20. When the glomerular filtration rate falls, BUN rises until constancy of their product is restored.[18] The marked reduction in urinary urea induced by the rhubarb extract may be regarded as reflecting a decrease in the glomerular filtration rate. However, administration of the rhubarb extract to rats produced a decrease in the serum urea nitrogen level, as reported previously.[1] The urea concentration in the liver was also dose-dependently decreased.[1] This decrease of urinary urea at the time when the serum urea nitrogen is declining supports the view that a large part of the effect may be due to decreased urea production rather than to depression of renal failure.

Moreover, it should be mentioned that no change was seen in the urinary excretion of creatinine throughout the experimental period (Table I). Creatinine excretion is almost exclusively dependent on the rate of filtration, since tubular reabsorption does not occur and only small quantities are secreted. For this reason, an abnormally elevated creatinine concentration in the serum is a more reliable indication of decreased renal function.[18] However, the decreased creatinine level in the serum which was found previously[1] seems inconsistent with the unchanged urinary output of creatinine.

Creatinine is produced from creatine and phosphocreatine in muscle. The quantity produced and excreted depends on skeletal muscle mass.[18] Thus, the decrease of serum creatinine by the rhubarb extract treatment may be related to a decrease of creatinine production in muscle. These effects are also regarded as reflecting a metabolic production of methylguanidine (MG) after treatment of uremic rats with the rhubarb extract. With regard to the metabolic origin of MG, Mikami and his co-workers demonstrated that most MG is produced through creatinine.[23] There is much evidence in support of MG as a potent uremic toxin. Giovannetti *et al.*[24] reported on the toxicity of MG and found various uremia-like symptoms. Our experimental results in the previous study[1] indicated that administration of rhubarb extract decreased the serum MG level of uremic rats in a dose-dependent manner, and MG was not detectable in the serum or in the kidney of the rhubarb extract-treated group given 55 mg/rat/d for 24 d. In view of the data on urea, creatinine, and other nitrogenous fragments including guanidino compounds such as MG, rhubarb extract may play a role in improving hyperazotemia.

On the other hand, as reported previously,[25] intraperitoneal administration of rhubarb extract markedly increased the urinary excretion of both urea and creatinine, indicating an improvement of renal clearance in the uremic state. Daily administration of rhubarb extract also caused marked reduction in serum urea nitrogen and creatinine.[26] Such observations are different from those obtained following oral administration. The effects of intraperitoneal and oral administration of rhubarb extract should be further studied in order to elucidate the

precise pharmacological activity.

In addition, a smaller effect on the urinary excretion of electrolytes was noted in rats of the rhubarb extract-treated group (Table II). Though further studies will be needed to elucidate the mechanism of the effect, published data from our laboratory showed that the renal histological changes were characterized by generation of tubular epithelia with dilation of the tubular lumina, deposits of amorphous birefringent crystals in the proximal and distal tubuli, and the formation of foreign body granuloma,[5-8,11,12] It seems likely that anomalies such as impairment of renal function and disturbance in the proximal tubules can be improved by treatment with rhubarb extract. The polyuria-preventive effect of rhubarb extract (Fig. 1) might also be explained partly by the effect on tubular reabsorption.

As reported previously,[3-12] hyperazotemia, accumulation of uremic toxins, metabolic imbalance of amino acids and electrolytes, hypoalimentary condition, hormonal imbalance, *etc.*, were induced in rats by feeding adenine, and they bore a close resemblance to the metabolic abnormalities noted in chronic renal failure in humans. Histologically, disorder in some distal urinary tubules with proximal urinary tubules as the center of disorder, secondary glomerular disturbance and in severe cases renal atrophy were found, indicating that the rats are available as a chronic renal failure model. The previous results[1] on serum constituents and the present experimental results indicated that rhubarb extract partly halted the progression of renal failure, and suggested that rhubarb extract may be useful for the therapy of chronic renal failure.

Following the experiments reported in the present paper, we observed that after chronic renal failure had been induced in rats by administering adenine, rhubarb extract treatment resulted in a significant reduction in serum urea nitrogen and guanidino compounds.[27] Furthermore, there has recently been a case report of a patient with chronic renal failure who responded well to rhubarb or rhubarb-containing prescriptions.[28]

References

1) T. Yokozawa, N. Suzuki, P. D. Zheng, H. Oura, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 4506 (1984).
2) A. E. Harper, *J. Nutr.*, **68**, 405 (1959).
3) T. Yokozawa, H. Oura, H. Nakagawa, and K. Takemoto, *J. Jpn. Soc. Food Nutr.*, **34**, 35 (1981).
4) T. Yokozawa, H. Oura, H. Nakagawa, and H. Fukuda, *Nippon Nôgeikagaku Kaishi*, **55**, 811 (1981).
5) T. Yokozawa, H. Oura, H. Nakagawa, and T. Okada, *Nippon Nôgeikagaku Kaishi*, **56**, 655 (1982).
6) T. Yokozawa, H. Oura, and T. Okada, *J. Nutr. Sci. Vitaminol.*, **28**, 519 (1982).
7) H. Oura, T. Yokozawa, M. Fukase, and F. Koizumi, *Igaku No Ayumi*, **125**, 791 (1983).
8) T. Yokozawa, H. Oura, P. D. Zheng, M. Fukase, F. Koizumi, and M. Kanaoka, *Agric. Biol. Chem.*, **47**, 1297 (1983).
9) T. Yokozawa, P. D. Zheng, and H. Oura, *Agric. Biol. Chem.*, **47**, 2341 (1983).
10) T. Yokozawa, P. D. Zheng, and H. Oura, *J. Nutr. Sci. Vitaminol.*, **30**, 245 (1984).
11) H. Oura, T. Yokozawa, P. D. Zheng, and F. Koizumi, *Igaku No Ayumi*, **130**, 729 (1984).
12) T. Yokozawa, H. Oura, and F. Koizumi, *Jpn. J. Nephrol.*, **27**, 371 (1985).
13) T. Nagasawa, S. Shibutani, and H. Oura, *Yakugaku Zasshi*, **99**, 71 (1979).
14) R. M. Archibald, *J. Biol. Chem.*, **157**, 507 (1945).
15) K. Nakamura, "Rinsyo Kagaku Bunseki," Vol. 2, ed. by M. Saito, M. Kitamura, and M. Niwa, Tokyo Kagaku Dojin, Tokyo, 1973, p. 53.
16) K. Samejima and M. Kitamura, "Rinsyo Kagaku Bunseki," Vol. 5, ed. by M. Kitamura, M. Saito, and M. Niwa, Tokyo Kagaku Dojin, Tokyo, 1973, p. 53.
17) T. Wajima, "Rinsyo Kagaku Bunseki," Vol. 5, ed. by M. Kitamura, M. Saito, and M. Niwa, Tokyo Kagaku Dojin, Tokyo, 1973, p. 116.
18) W. E. Rutherford, "Renal and Electrolyte Disorders," ed. by S. Klahr, Arco Publishing Company, New York, 1978, p. 101.
19) W. E. Mitch, E. Abras, and M. Walser, *Kidney Int.*, **22**, 48 (1982).
20) W. E. Mitch, V. U. Collier, and M. Walser, "Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids," ed. by M. Walser and J. R. Williamson, Elsevier/North-Holland, New York, 1981, p. 587.

21) R. T. Schimke, *J. Biol. Chem.*, **237**, 459 (1962).
22) S. Ratner, "Advances in Enzymology," Vol. 39, ed. by A. Meister, John Wiley and Sons, New York, 1973, p. 1.
23) H. Mikami, Y. Orita, A. Ando, M. Fujii, T. Kikuchi, K. Yoshihara, A. Okada, and H. Ando, "Urea Cycle Diseases," ed. by A. Lowenthal, A. Mori, and B. Marescau, Plenum Publishing Corporation, New York, 1983, p. 449.
24) S. Giovannetti, M. Biagini, P. L. Balestri, R. Navasesi, P. Giagnoni, A. de Matteris, P. Ferro-Milone, and C. Perfetti, *Clin. Sci.*, **36**, 445 (1969).
25) T. Yokozawa, P. D. Zheng, H. Oura, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 205 (1984).
26) T. Yokozawa, P. D. Zheng, H. Oura, M. Fukase, F. Koizumi, and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 2762 (1983).
27) T. Yokozawa, N. Suzuki, I. Okuda, H. Oura, and I. Nishioka, *J. Med. Pharm. Soc. for WAKAN-YAKU*, in press.
28) T. Mitsuma, K. Terasawa, T. Yokozawa, and H. Oura, *J. Med. Pharm. Soc. for WAKAN-YAKU*, **1**, 266 (1984).