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# Effect of rhubarb extract in rats with chronic renal failure under protein loading

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**Abstract**

The effects of rhubarb extract administration were investigated in rats with chronic renal failure under protein loading. Rats given rhubarb extract showed a significant decrease in the blood levels of urea nitrogen, creatinine and guanidinosuccinic acid, and a tendency toward a decreased methylguanidine blood level. Accumulation of nitrogen compounds in the hepatic and renal tissues was reduced after administration. The mechanism by which the progression of uremia is delayed by rhubarb extract under the conditions resulting from a high-protein diet is discussed on the basis of the present results.

**Key words** rhubarb, chronic renal failure, protein loading, rat

## Introduction

A low-protein diet is widely used as a form of therapy for chronic renal failure. However, in addition to dysbolism of amino acid and the protein characteristics of renal failure, a low-protein diet often induces both protein and amino acid deficiency, resulting in a negative nitrogen equilibrium or protein catabolism in the body.[1] Therefore, a new therapy superior to the usual conservative therapy, for slowing down the progression of renal failure itself through improvement of protein malnutrition, is desirable. The authors previously carried out daily oral administration of rhubarb extract to rats with adenine-induced chronic renal failure and reported the occurrence of dose-dependent anti-uremic effects including improvement of hyperazotemia, a decrease or absence of strong uremic toxins such as methylguanidine and guanidinosuccinic acid in the blood, an improved serum amino acid pattern, and improvement of hypocalcemia and hyperphosphatemia.[2-4] Also, clinical therapy using rhubarb or traditional Chinese prescriptions containing rhubarb has occasionally been tried, and its usefulness now established.[5,6] In the present study, in order to examine whether rhubarb extract was able to extend the limit of diet therapy, the effects of this extract under the conditions resulting from protein loading applied for the purpose of improving malnutrition were investigated.

## Materials and Methods

*Animals and treatment* : Male Wistar rats initially weighing about 150 g were used. The animals were divided into three groups, *i.e.,* one given a diet containing 18% casein, another given a diet containing 40% casein (control) and the other given rhubarb extract and a diet containing 40% casein. To these diets, adenine was added at a level of 0.75 g/100 g of the diet. Renal failure was gradually induced with time following the start of adenine feeding, as reported previously.[7-10] Rhubarb extract was allowed *ad libitum* at a concentration of 1 mg/ml in drinking water, while control rats received tap water. The dose of rhubarb extract was about 25 mg/rat/day

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during the experimental period. Food was given by pair-feeding to obtain a constant intake, and equal amounts of ingestive components other than protein were given to the three groups. On the 24th day of the feeding period, rats were stunned by a sharp blow to the head. Blood was collected into conical centrifuge tubes and the serum was separated by centrifugation immediately after blood collection. The liver and kidney were removed quickly, cooled on ice, and weighed rapidly.

*Preparation of rhubarb extract* : Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted with water at 100℃, as described previously.[2] The aqueous extract was filtered through 4 layers of gauze and the filtrate was freeze-dried under reduced pressure to obtain a brown residue with a yield of about 25%.

*Determination of urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid and albumin in serum* : Urea nitrogen and albumin were determined using commercial reagents (BUN KAINOS obtained from Kainos Laboratories, Inc., Tokyo, Japan ; Albumin B-Test Wako from Wako Pure Chemical Industries, Ltd., Osaka, Japan). For determination of creatinine, methylguanidine and guanidinosuccinic acid, the serum was deproteinized by addition of trichloroacetic acid (final concentration, 10%). The supernatant obtained by centrifugation at 3000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system. A fluorescence spectrometer (excitation 365 nm, emission 495 nm ; model FP-210, Japan Spectroscopic Co., Tokyo, Japan) was used for detection of the substances on the column.

*Determination of urea in liver and kidney* : A portion of the tissue was homogenized with 9 volumes of ice-cold water in a Potter-Elvehjem glass homogenizer. Part of the homogenate was diluted with ice-cold water and deproteinized by the addition of 0.15 M barium hydroxide and 5% zinc sulfate. After centrifugation at 3000 rpm for 15 min, urea in the supernatant obtained was determined by the procedure of Archibald.[11]

*Determination of methylguanidine and guanidinosuccinic acid in liver and kidney* : The other parts of the liver and kidney samples were homogenized with trichloroacetic acid (final concentration, 10%) and the supernatant obtained by centrifugation at 3000 rpm for 10 min was applied to a Japan Spectroscopic liquid chromatograph.

*Statistics* : The significance of differences between the rats given an 18% casein diet and those given an 40% casein diet (control or rhubarb extract-treated group) was tested by means of Student's $t$ test.

## Results

Table I shows variations in serum constituents. With the high-protein (40% casein) diet, urea nitrogen was significantly increased by 42%, and creatinine and methylguanidine were

Table I Effect of rhubarb extract on serum constituents.

| | Urea-N (mg/dl) | Cr (mg/dl) | MG (μg/dl) | GSA (μg/dl) | Albumin (g/dl) |
|---|---|---|---|---|---|
| 18% Casein diet | 108.2± 9.5<br>(100) | 2.84±0.10<br>(100) | 7.00±0.35<br>(100) | 90.09± 8.74<br>(100) | 3.41±0.10<br>(100) |
| 40% Casein diet | | | | | |
| Control | 153.3± 5.8<br>(142)**<br>[100] | 1.72±0.15<br>(61)***<br>[100] | 1.98±0.93<br>(28)***<br>[100] | 88.69±13.35<br>(98)<br>[100] | 3.59±0.12<br>(105)<br>[100] |
| Rhubarb extract | 114.4±17.7<br>(106)<br>[75]# | 1.22±0.06<br>(43)***<br>[71]# | 1.16±0.75<br>(17)***<br>[59] | 47.54± 6.49<br>(53)**<br>[54]# | 3.56±0.08<br>(104)<br>[99] |

Urea-N, urea nitrogen ; Cr, creatinine ; MG, methylguanidine ; GSA, guanidinosuccinic acid. Values are means±S.E. for 5 or 6 rats. Figures in parentheses are percentages of the 18% casein diet or 40% casein diet value. #Significantly different from the 18% casein diet or 40% casein diet value, $p<0.05$, **$p<0.01$, ***$p<0.001$.

significantly decreased by 39% and 72%, respectively. There were no significant variations in guanidinosuccinic acid, whereas albumin tended to be increased. In rats given rhubarb extract (25 mg/rat/day) for 24 consecutive days during high-protein ingestion, the urea nitrogen level fell to 114.4 mg/dl (significantly decreased by 25% of the control value). The rats treated with rhubarb extract showed a significant decrease in creatinine from 1.72 to 1.22 mg/dl. The data in Table I further indicate that the rhubarb extract-treated group showed a significant decrease in guanidinosuccinic acid. The methylguanidine level showed a tendency toward a decrease. Determination of levels of nitrogen compounds in the tissues revealed a significant elevation of urea, a tendency toward an increase of guanidinosuccinic acid and a decrease of methylguanidine in the liver after the high-protein diet (Table II). In contrast, methylguanidine was completely eliminated when rhubarb extract was administered. The guanidinosuccinic acid level in the liver was decreased from 9.61 μg/g tissue to 4.02 μg/g tissue. Urea content also showed a moderate decrease, but this was not statistically significant. In the kidney, the high-protein diet caused a significant elevation of urea and a tendency toward an increase in guanidinosuccinic acid. The content of methylguanidine behaved differently from those of the other two nitrogen compounds, decreasing significantly by 50% (Table III). Administration of rhubarb extract to rats resulted in a significant decrease of guanidinosuccinic acid ; as shown in Table III, the guanidinosuccinic acid content was 18% less in the rhubarb extract-treated group than in the

Table II Effect of rhubarb extract on hepatic constituents.

| | Urea (mg/g tissue) | MG (μg/g tissue) | GSA (μg/g tissue) |
|---|---|---|---|
| 18% Casein diet | 1.60±0.09<br>(100) | 0.244±0.028<br>(100) | 5.19±1.22<br>(100) |
| 40% Casein diet | | | |
| Control | 2.65±0.13<br>(166)**<br>〔100〕 | 0.127±0.082<br>(52) | 9.61±2.46<br>(185)<br>〔100〕 |
| Rhubarb extract | 2.28±0.41<br>(143)<br>〔86〕 | N.D. | 4.02±0.39<br>(77)<br>〔42〕# |

MG, methylguanidine ; GSA, guanidinosuccinic acid. Values are means±S.E. for 5 or 6 rats. Figures in parentheses are percentages of the 18% casein diet or 40% casein diet value. #Significantly different from the 18% casein diet or 40% casein diet value, $p<0.05$, $^{**}p<0.001$.

Table III Effect of rhubarb extract on renal constituents.

| | Urea (mg/g tissue) | MG (μg/g tissue) | GSA (μg/g tissue) |
|---|---|---|---|
| 18% Casein diet | 2.40±0.22<br>(100) | 0.328±0.031<br>(100) | 2.58±0.49<br>(100) |
| 40% Casein diet | | | |
| Control | 4.21±0.17<br>(175)***<br>〔100〕 | 0.164±0.025<br>(50)**<br>〔100〕 | 3.59±0.30<br>(139)<br>〔100〕 |
| Rhubarb extract | 3.61±0.32<br>(150)**<br>〔86〕 | 0.120±0.020<br>(37)***<br>〔73〕 | 2.95±0.19<br>(114)<br>〔82〕# |

MG, methylguanidine ; GSA, guanidinosuccinic acid. Values are means±S.E. for 5 or 6 rats. Figures in parentheses are percentages of the 18% casein diet or 40% casein diet value. #Significantly different from the 18% casein diet or 40% casein diet value, $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

control group. A decrease in urea and methylguanidine was also observed in the rhubarb extract-administered group ; however, these changes were not statistically significant.

## Discussion

The authors previously reported that administration of rhubarb extract combined with a 18% protein diet reduced the accumulation of nitrogen compounds, such as urea nitrogen, creatinine and guanidino compounds, which had been increased in the blood due to renal failure.[2-4] These findings suggested that rhubarb administration allows some degree of protein loading to occur, facilitating improvement of the general condition, thus indicating the usefulness of this regimen as a conservative therapy for chronic renal failure. The present study examined the effects of rhubarb extract administration under the conditions resulting from a high-protein diet. It was found that rats given rhubarb extract showed a significant decrease in the blood levels of urea nitrogen, creatinine and guanidinosuccinic acid and a tendency toward a decrease in the blood level of methylguanidine, all of which are known to show high levels of accumulation in renal failure. In addition, accumulation of nitrogen compounds in the hepatic and renal tissues was reduced after administration, suggesting that rhubarb administration can slow down the progression of uremia even under high-protein loading. On the other hand, despite the fact that the protein intake from the high-protein diet used in the present study was about 2.2 times higher than that from the 18% protein diet (the blood urea nitrogen level was about 1.4 times higher with the high-protein diet), the blood levels of creatinine and methylguanidine were both decreased significantly. The methylguanidine levels in the liver and kidney, the main organs producing methylguanidine as reported previously,[9] were significantly decreased or tended to be decreased. Therefore, a high-protein diet alone seems to be able to delay the progression of uremia, though to a lesser extent than in combination with rhubarb extract. There have been few reports on variations in guanidino compounds after high-protein feeding ; the report by Orita *et al.*[12] is the only document available. They studied the effects of a high-protein diet in rats with chronic renal failure prepared by the method of Platt *et al.*[13] According to their report, in rats given a high-protein diet, the serum methylguanidine level tended to be increased, the methylguanidine content in the liver was significantly increased by 18%, and corresponding levels in other organs and urinary methylguanidine secretion were unchanged. However, their data were obtained from rats with renal failure developed as a result of a decrease in the number of functional nephrons, whereas our data were obtained from rats with chronic renal failure caused by disturbance in the proximal tubules and, in part, a decrease in the number of glomeruli. In addition, unlike our study, they employed free ingestion. Therefore, there is a possibility that other ingestive components might have affected their results. The present authors have previously observed that, in adenine-fed rats, the levels of glomerular filtration rate, renal plasma flow and renal blood flow after a high-protein diet were approximately double those after a 18% protein diet, demonstrating a state of increased renal function (unpublished data). This finding seems to indicate that low levels of uremic toxins including methylguanidine in the body resulted from active urinary excretion of such substances. In recent years, the hyperfiltration theory based on data from animal experiments has become dominant, and the relation between hyperfiltration and progression of renal disease, particularly glomerulosclerosis, has been pointed out by Brenner *et al.*[14] According to this theory, an increase in ingested protein causes an increase in glomerular filtration, leading to glomerulosclerosis. From the results of the present study, protein ingestion and rhubarb extract administration are effective to some extent for improving amino acid and protein metabolism, judging from the serum albumin level. However, the role of rhubarb extract in the process of change from increased glomerular filtration and renal blood flow to the terminal state, glomerulosclerosis, remains to be elucidated.

## 和文抄録

腎不全ラットに蛋白質を負荷した状態における大黄エキスの効果を検討した。血中成分では尿素窒素，クレアチニン，グアニジノコハク酸がいずれも有意に低下し，メチルグアニジンも低下傾向を示した。また肝，腎組織中の窒素化合物の蓄積も軽減し，高蛋白摂取下でも大黄は尿毒症の進行を遅延させ得る可能性が示唆された。

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