Differences in cathartic action among different types of the Oriental drug rhubarb

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Abstract

Three different types of rhubarb : rhubarb A (produced in the Province of Si-Chuan (四川), China), B (cultivated and processed in Japan) and C (tablets manufactured with the processed rhizome of *Rheum palmatum* L. from the Province of Qing-Hai (青海), China) were administered to 12 healthy male volunteers for three days each, and their quality was evaluated in terms of the number of bowel movements, bowel sounds, urinary volume and various blood chemical parameters. Upon overall assessment of these parameters, processed rhubarb, with a weaker cathartic action, is suitable for therapeutic use in patients with chronic renal failure.

Key words rhubarb, sennoside, tannin, catharsis, man, rat, chronic renal failure.

Introduction

Evaluation of the quality of Oriental drugs has long been dependent on the color, shape, taste, smell, and place of production of the crude drugs and empirical judgement. However, with the aim of establishing the status of these Oriental drugs as medicines, experimental and clinical studies along with determination of the active constituents mainly responsible for their biological and pharmacological activity have made it possible to evaluate their quality on a scientific basis.

Commercial rhubarb "Ya-Huang (雅黄)" from China has been used mainly for preparing rhubarb prescriptions in Oriental medicine. Since sennoside contained in rhubarb has been shown to be responsible for its cathartic action,[1] production of rhubarb of better quality has been attempted using the sennoside content as an index, and modern rhubarb drugs are now widely available commercially. However, in Oriental medicine, clinical application of rhubarb is not aimed at catharsis alone. As Shen-Nong-Ben-Cao-Jing (神農本草経), a classic textbook of Chinese medicine, mentions that "rhubarb eliminates disturbance of the vascular system, has diuretic and cathartic actions, and promotes metabolism through improvement of gastrointestinal function and harmonization of visceral organs," the need to investigate the pharmacological actions of rhubarb other than catharsis has long been acknowledged.

We have developed a simple animal model of experimental renal failure in the rat.[2,3] Using this model, we have demonstrated an anti-uremic action of rhubarb[4-6] and found that rhubarb and Wen-Pi-Tang (温脾湯) improve uremia in patients with renal failure.[7,8] In this regard, research on evaluation of the quality of rhubarb was initiated to produce rhubarb having a weaker cathartic action. Such demand is derived from the known fact that, in patients with renal failure, a strong cathartic action may worsen the existing disorder of water and electrolyte metabolism, leading to fatal damage.[9] In the present study,

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differences in the pharmacological action among three types of rhubarb were investigated in healthy volunteers.

Materials and Methods

Types of rhubarb used and their preparation : The following three types of rhubarb were used : rhubarb A (produced in the Province of Si-Chuan, China, and purchased from Tochimoto Tenkaido, Co., Ltd., Osaka, Japan), B (the rhizome of *Rheum coreanum* introduced from Kiyosato in Nagano Prefecture, Japan, and subcultured in the herb garden of our university) and C (tablets manufactured with the rhizome of *Pheum palmatum* L. from the Province of Qing-Hai, China, each tablet containing 0.3 g of rhubarb).

Qualitative analysis of sennosides A and B, and tannins in rhubarbs : For analysis of sennosides A and B, each rhubarb sample (50 mg) was finely pulverized and extracted overnight with tetrahydrofuran (THF) (1 ml) at room temperature. The extract was filtered through a Millipore membrane, and the filtrate (5 μl) was injected into an HPLC apparatus. For analysis of tannins, the rhubarb (50 mg each) was extracted with a mixture of water and THF (2 : 3) (5 ml) and the extract was treated in a similar way to that described above.

Conditions for HPLC analysis : HPLC was performed on a Toyo Soda apparatus equipped with a CCPM solvent delivery system, a UV-8 model II spectrometer and a Cosmosil 5Ph column (Nakarai Chemical Co., Ltd.) (4.6 mm i.d.×250 mm) [column temperature : 40°C and flow rate : 0.8 ml/min]. Sennosides A and B were analyzed by gradient elution with an aqueous solution of 50 mM H_3PO_4 containing increasing proportions of CH_3CN [15→25 % (0→40 min), 25→80 % (40→50 min), 80 % (50→70 min), while tannins were eluted with a mixture of 50 mM H_3PO_4 aqueous solution and CH_3CN [15→25 % (0→30 min), 25→45 % (30→50 min), 45 % (50→60 min), 45→80 % (60→70 min), 80 % (70→90 min)]. Detection was achieved at wavelengths of 340 nm and 280 nm for sennosides and tannins, respectively.

Administration to healthy volunteers : Twelve men aged 19~23 years were allocated to 6 groups, and given two divided doses of rhubarbs A and B (a daily dose of 1.2 g was decocted in boiling water for 1 h and adjusted to 100 ml) or rhubarb C (4 tablets/day) daily for three consecutive days. In each group, the subjects were given the three types of rhubarb in turn, changing the drug every three weeks. Changes in subjective symptoms and clinical laboratory data were observed for one week from the beginning of drug administration for each course of administration. Subjective symptoms were assessed on the day before administration (day 0), on days 1, 2 and 3 of administration, the day after the end of administration (day 4), and 4 days after the end of administration (day 7) by means of an interview sheet. A total of 201 items of subjective symptoms were rated using five grades ("no" [no symptoms], 0 point ; "very slight," 1 ; "slight," 2 ; "considerable," 3 ; "marked,"4). The number of bowel movements per day was recorded. Fasting morning blood and urine samples were collected on days 0, 4 and 8, and subjected to laboratory tests. All the volunteers were placed on the duration of follow-up for at least 1 week before the study was initiated. Informed consent was obtained from the volunteers prior to enrollment in the trial.

Animal experiment : Male rats of the Wistar strain, with a body weight of 200-210 g, were kept in an animal room at an ambient temperature of 23± 1°C under a 12 h dark-light cycle. They were allowed an adaptation period of several days, during which they were fed on a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan). They were then fed *ad libitum* on an 18 % casein diet containing 0.75 % adenine, which produced experimental renal failure. In rats with renal failure induced by adenine, it has been confirmed previously, both histologically and biochemically, that the renal failure progresses as the period of adenine feeding is prolonged.[2,3] During the adenine feeding period, an aqueous solution of rhubarb A, B or C extract was dissolved in water, and given to rats orally every day as drinking water. The dose (125 mg/kg body weight) was adjusted by regulating the concentration in relation to water consumption. The extract was obtained by gently heating 100 g of each rhubarb under reflux in 1000 ml of water for 60 min, and about 500 ml of decoction was obtained. The extract was then concentrated under

reduced pressure to leave a residue. Control rats were given a corresponding amount of water. The rats were killed on day 25 by decapitation. Blood was collected into a conical centrifuge tube, and the serum was separated immediately by centrifugation. Throughout the experimental period, there were no statistically significant differences between the controls and rats treated with extract with regard to changes in body weight. The food intake of each rat was essentially proportional to weight change. Urea nitrogen was determined using commercial reagents (BUN Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan). Creatinine (Cr), methylguanidine (MG) and guanidinosuccinic acid (GSA) were measured using a Japan Spectroscopic liquid chromatograph with a step-gradient system by the method of Higashidate *et al.*[10] A fluorescence spectrometer, model FP-210 (excitation 365 nm, emission 495 nm ; Japan Spectroscopic Co., Tokyo, Japan), was used for the detection of Cr, MG and GSA on the column. Six rats were used for each experimental group.

Statistics : Values are expressed as means±S.E. Statistical analysis was performed by Dunnett's test.

Results

Component analysis : Although all of rhubarbs A, B and C contained relatively large amounts of tannin, rhubarb C contained almost no sennosides A and B, as shown in Fig. 1 and 2.

Subjective symptoms : Symptoms probably due to the cathartic action of rhubarb, such as diarrhea and hyperactive bowel sounds, were most frequent. As shown in Fig. 3, the mean number of bowel movements was increased during the period of administration of both rhubarb A and B (particularly rhubarb B). Two men given rhubarb A and 5 given rhubarb B had 4 or more bowel movements per day for 3 days, while no such effect was observed in the group given rhubarb C. More specifically, in the rhubarb C group, 11 of the 12 subjects had 2 or fewer bowel movements per day. In the rhubarb B group, hyperactive bowel sounds were more marked, and diarrhea was more frequent than in the other courses. With regard to the overall impression of the cathartic actions of the three kinds of rhubarb, 8 of the 12 subjects reported that the

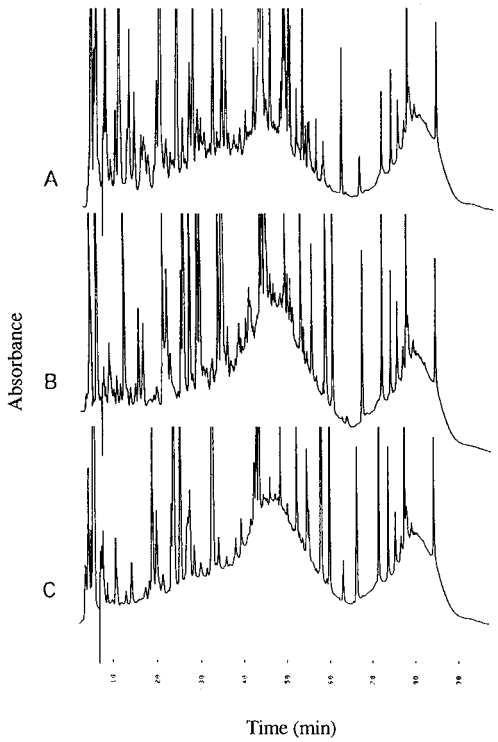

Fig. 1 Elution pattern of tannin fraction in rhubarbs A, B and C.
A, rhubarb A ; B, rhubarb B ; C, rhubarb C.

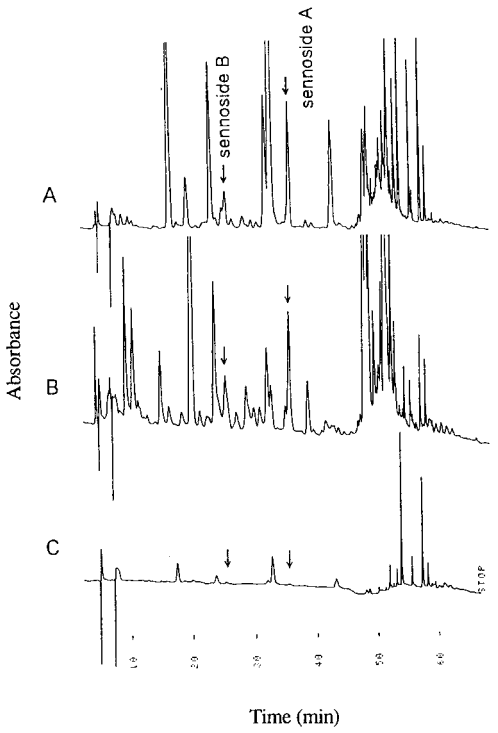

Fig. 2 Elution pattern of sennoside A and B in rhubarbs A, B and C.
A, rhubarb A ; B, rhubarb B ; C, rhubarb C.

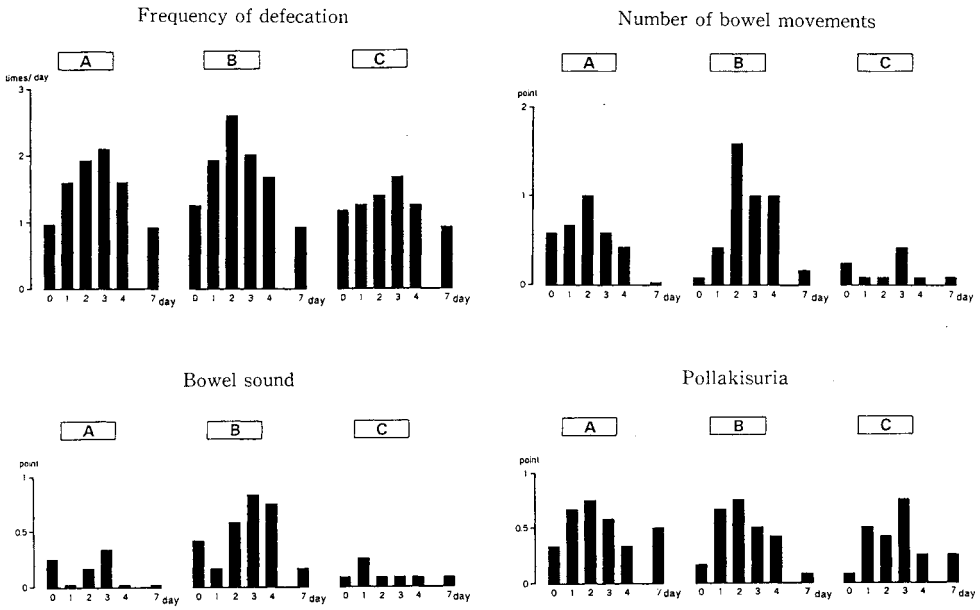

Fig. 3 Changes in subjective symptoms after administration of rhubarbs A, B and C. A, rhubarb A ; B, rhubarb B ; C, rhubarb C.

descending order of potency was rhubarb B, A and C, and 10 reported that rhubarb B was most potent. Three subjects noted an obvious increase in urinary volume after taking all the rhubarb types. Analysis of the interview sheets of all 12 subjects suggested a tendency for an increased frequency of urination during the administration of any type of rhubarb.

Clinical laboratory data : There were no apparent abnormalities of blood chemical parameters caused by administration of any rhubarb, and only slight variations were noted during the observation period, as shown in Table I. The blood urea nitrogen (BUN) level tended to decrease on day 4 in subjects given rhubarbs A and C, while it tended to increase in those given rhubarb B, showing a significant difference between rhubarbs B and C on day 4. There was a similar tendency of variation in the uric acid level, but any differences were not significant. No particular variation was found in the creatinine (Cr) level. The levels of inorganic phosphorus (iP) in subjects given rhubarbs A and B showed a pattern similar to that of urea nitrogen, with a significant difference between the two rhubarbs. The calcium (Ca) level tended to decrease in subjects given any rhubarb type during the observation period, a significant difference being evident between day 0 and day 4 for rhubarb A and

Table I Clinical laboratory data.

Item	Day	Rhubarb A	Rhubarb B	Rhubarb C
BUN (mg/dl)	0	13.6±1.3	13.0±0.9	12.8±0.7
	4	12.5±0.7	14.4±0.7	12.0±0.7
	8	13.7±1.1	13.6±1.0	12.9±0.8
Uric acid (mg/dl)	0	5.6±0.4	5.5±0.4	5.4±0.4
	4	5.3±0.3	5.7±0.3	5.3±0.4
	8	5.5±0.3	5.5±0.4	5.5±0.3
Cr (mg/dl)	0	1.0±0.1	1.0±0.1	1.0±0.1
	4	1.0±0.1	1.0±0.1	1.0±0.1
	8	1.0±0.1	1.0±0.1	1.0±0.1
iP (mg/dl)	0	3.7±0.2	3.6±0.2	3.5±0.2
	4	3.5±0.1	3.9±0.1[a]	3.6±0.1
	8	3.7±0.1	3.7±0.2	3.7±0.1
Ca (mg/dl)	0	4.28±0.06	4.23±0.05	4.20±0.06
	4	4.13±0.04[c]	4.16±0.06	4.16±0.05
	8	4.13±0.05[c]	4.13±0.02[b]	4.10±0.05[a]
T-Ch (mg/dl)	0	164.8±6.5	164.5±5.0	165.3±5.7
	4	164.3±7.7	168.6±5.8	162.9±5.3
	8	164.6±7.5	163.8±4.0	164.4±7.3
HDL-C (mg/dl)	0	49.2±3.3	48.5±2.7	49.0±2.0
	4	50.8±3.7	50.0±2.7	50.2±2.2
	8	49.3±3.0	51.3±2.7	49.4±1.9
GOT (U)	0	14.2±1.2	12.8±0.7	15.0±1.2
	4	12.5±0.7	12.8±1.2	13.2±0.8[a]
	8	14.7±1.1	13.2±0.8	13.9±1.0
GPT (U)	0	11.8±1.3	9.8±0.6	11.2±1.1
	4	10.5±0.8	9.9±0.8	11.0±0.7
	8	11.3±1.1	9.7±0.6	11.3±0.8

Table I (continued)

Item	Day	Rhubarb A	Rhubarb B	Rhubarb C
ALP (U)	0	171.7±11.5	173.8±11.6	178.4±11.7
	4	169.8±11.9	171.7±10.6	173.8±12.4
	8	168.9±11.6	172.8±11.5	178.2±11.9
LDH (U)	0	312.3±14.2	313.3±23.4	326.8±19.8
	4	284.1±9.8[a]	311.7±17.8	295.2±14.1[a]
	8	306.8±20.2	333.4±18.7	312.1±14.7
RBC ($\times 10^4$/cmm)	0	513.3±9.6	504.4±9.8	506.5±9.0
	4	513.3±7.7	509.1±9.6	510.1±13.0
	8	505.4±10.6	509.9±10.6	512.7±11.4
WBC (/cmm)	0	6142±634	6536±689	6573±644
	4	6233±481	6483±496	6008±378
	8	5700±541	6367±439	6342±653
Ht (%)	0	45.8±0.8	44.9±0.9	45.3±0.8
	4	46.0±0.8	45.8±0.8	45.8±1.0
	8	45.2±0.8	45.7±0.8	45.8±1.0
Hb (g/dl)	0	15.8±0.3	15.5±0.4	15.6±0.3
	4	15.9±0.3	15.7±0.4	15.7±0.4
	8	15.6±0.3	15.7±0.4	15.8±0.4

Significantly different from the pretreatment value : [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$.

between day 0 and day 8 for rhubarbs A, B and C. The degree of variation, however, was small in comparison with iP. The high-density lipoprotein-cholesterol (HDL-C) level was higher on day 4 than on day 0 in subjects given all three rhubarbs. The glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) levels tended to decrease on day 4 in subjects given rhubarbs A and C, while there was no such apparent tendency in those given rhubarb B, similarly to the variation in BUN. There were no particular variations in total cholesterol (T-Ch), alkaline phosphatase (ALP), red blood cell count (RBC), white blood cell count (WBC), hematocrit (Ht) or hemoglobin (Hb) during the observation period for any rhubarb.

Animal experimental data : The results of administration of each rhubarb are shown in Table II. The administration of rhubarb extract to rats resulted in a decrease in BUN ; rhubarb A produced a 27 % decrease, rhubarb B a 20 % decrease and rhubarb C a 27 % decrease, all being statistically significant. Similar changes produced by rhubarb administration were observed in the level of Cr. In addition, the MG values were significantly lower, by 53 %, 34 % and 50 %, in rats given rhubarbs A, B and C, respectively. The GSA values were also markedly and significantly decreased in rats given rhubarb in comparison with the control group. In rats given rhubarbs A and B, however, diarrheal symptoms were observed.

Discussion

Although rhubarb exerts a metabolism-improving effect at a dose that does not cause diarrhea in patients with chronic renal failure,[7,8] some patients are so susceptible to the cathartic action of rhubarb that it may be difficult to continue its administration. To weaken the cathartic activity, heat processing of rhubarb to reduce its sennoside content has been attempted. Rhubarb C used in the present study is one of these products, and rhubarbs A and B were prepared by boiling for 1 h, a longer period than the usual decocting time. As a result, the cathartic effect of rhubarb B was most potent, followed by rhubarb A, and that of rhubarb C was weakest. The results of blood chemical tests revealed that the levels of urea nitrogen, uric acid, and inorganic phosphorus, which are high in chronic renal failure, tended to decrease in

Table II Effect of various rhubarb extracts on serum constituents in rats with renal failure.

Group	BUN (mg/dl)	Cr (mg/dl)	MG (μg/dl)	GSA (μg/dl)	iP (mg/dl)	Ca (mg/dl)
Control	121.3±8.0	3.17±0.22	12.14±1.11	101.39±6.88	15.72±0.61	8.10±0.12
Rhubarb A	88.4±4.0[b]	2.39±0.15[a]	5.66±0.39[c]	68.94±6.31[b]	11.01±0.57[c]	9.31±0.43[a]
Control	114.4±7.2	3.11±0.11	11.74±1.15	98.50±7.39	14.71±0.58	8.25±0.39
Rhubarb B	91.0±6.7[a]	2.70±0.13[a]	7.73±0.76[a]	71.65±5.08[a]	12.10±0.64[a]	8.74±0.65
Control	122.8±8.4	3.28±0.21	11.52±0.77	94.79±5.30	15.40±0.57	8.06±0.06
Rhubarb C	89.3±3.4[b]	2.54±0.11[a]	5.72±0.57[c]	57.31±4.02[c]	10.56±0.68[c]	9.11±0.28[b]

Significantly different from the control value : [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$.

subjects given rhubarb A on day 4, i.e., the day after the end of administration. In contrast, the corresponding levels increased or tended to increase in subjects given rhubarb B. Rhubarb C showed a pattern variation similar to, but not as apparent as, that of rhubarb A. Although catharsis was conspicuous in subjects given rhubarb B, there was no particular variation in hematocrit during the observation period after administration of any rhubarb type, indicating no involvement of hemoconcentration (dehydration) in the variations of biochemical parameters.

These findings were obtained in healthy subjects, and therefore cannot readily be extrapolated to patients with chronic renal failure. However, the present results suggest that rhubarb A or C is more appropriate than rhubarb B as a drug used for treatment of uremia. However, unlike the clinical data, administration of rhubarbs A, B and C to rats showed an effect against uremic toxins, achieving a significant decrease in MG, which is currently known to be the most potent uremic toxin. This suggests that rhubarb has the potential to improve the condition of renal failure. In view of the present situation where only a few medicaments are available for the treatment of chronic renal failure, we think that our data indicate the usefulness of rhubarb for the treatment of this condition. However, the diarrheal symptoms produced by rhubarbs A and B were marked, suggesting that rhubarb C is most suitable for therapeutic use in patients with chronic renal failure.

The clinical laboratory data showed that the levels of GOT, GPT and LDH tended to decrease, particularly in subjects given rhubarb A, while there was no such tendency in subjects given rhubarb B. The pattern of variation in the action of rhubarb C was intermediate between those of rhubarbs A and B. Although its relevance remains unclear, rhubarbs A and B also showed a distinct difference, while rhubarb C exhibited an intermediate action, in terms of BUN. The recordings of subjective symptoms suggested that a diuretic effect was achieved with any of the rhubarbs A, B and C, consistent with descriptions suggesting a water-eliminating (diuretic) action of rhubarb in classic textbooks of Chinese medicine, such as Shen-Nong-Ben-Cao-Jing, thus indicating a theme for future investigation.

Since it is rare to use rhubarb as monotherapy (one exception is the use of rhubarb in Shogun-to), related statistical data are currently insufficient. In this regard, the results of the present study serve as fundamental material for considering future clinical applications. However, the fact that the rhubarb product manufactured in China proved to have the weakest cathartic action suggests that improvement not only in the quality of rhubarb but also in its processing is necessary in the future.

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和文抄録

健常男子12名に対し3種類の大黄［雅黄（A），富山県産大黄（B），大黄炮製品錠剤（C）］を3日間投与し，その品質評価について，排便回数，腹部蠕動音，尿量並びに各種血液検査を行い比較検討した。本実験結果は今後の臨床応用の基礎資料となるものと考えられるが，修治大黄は瀉下作用が弱く，慢性腎不全における治療目的に有用である可能性が示唆された。

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