ORIGINAL

Effects of single administration of Rokumi-gan (TJ-87) on serum amino acid concentration of 6 healthy Japanese male volunteers

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Abstract : Rokumi-gan (TJ-87) has beneficial effects on renal diseases, including pollakisuria, dysuria and edema. We previously reported that its long-term administration clinically improved serum protein concentration and edema in renal failure. In this study, we focused on amino acid/protein contents in Rokumi-gan as one of its effectors. Commercially prepared Rokumi-gan contained arginine, aspartate and glutamate at the high levels, alanine, phenylalanine and serine at the moderate levels, and glycine, histidine, isoleucine, leucine, lysine and valine at the low levels. To examine effects of Rokumi-gan on serum amino acid concentrations, 6 healthy Japanese volunteers were treated with commercially prepared Rokumi-gan, an amino acid mixture, and lactose. In subjects treated with an amino acid mixture containing similar amounts of amino acids in Rokumi-gan (10 g), or lactose, serum amounts of many amino acids, except for arginine, gradually and significantly decreased until 6 hr after their treatments. In contrast, a single treatment with Rokumi-gan (10 g) increased serum levels of several amino acids, alanine, arginine, glutamate, glycine and serine. Serum concentrations of almost of all tested amino acids showed the peak value 1-2 hr after administration, and they were sustained at the basal level even 6 hr after the treatment. Our present results suggest that Rokumi-gan may be a beneficial amino acid supplier, because it could sustain serum amino acid concentration at the higher level than an amino acid mixture supplement. J. Med. Invest. 54 : 91-98, February, 2007

Keywords **:** *human volunteers, Rokumi-gan (TJ-87), serum amino acid*

INTRODUCTION

Rokumi-gan (TJ-87) is a plant prescription for asthenia of kidney in traditional East-Asian medicine (1). It consists of six plants (Rehmannia Root, Cornus Fruit, Dioscorea Rhizome, Alisma Rhizome, Hoelen and Moutan Bark) containing many low and high molecular compounds, such as terpenoids, flavonoids, amino acids and proteins (1-3). Rokumi-gan is one of the most common body enrichment drugs and is widely used for pollakisuria, dysurea and edema, which resulted from nephrosis and chronic nephritis (2-4). We also reported that a long-term administration of Rokumi-gan increased serum total protein concentration and improved edema in patients with renal failure (5). In contrast, it is well-known that supplementation of amino acids has beneficial effects on plasma amino acids and protein levels in chronic renal failure, leading to the improvement of dysuria and edema (6-8). Based on these findings,

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we hypothesized that Rokumi-gan may have several amino acids and/or proteins for its beneficial effects on renal diseases.

In this study, we examined amounts of amino acids in Rokumi-gan and its effect on serum amino acid concentration in 6 healthy volunteers to elucidate the function as a beneficial amino acid supplier. We found that Rokumi-gan contained high amounts of various amino acids, especially arginine, glutamate and aspartate. A single administration of Rokumi-gan effectively increased serum concentration of alanine, arginine, glutamate, glycine and serine in human beings, compared with treatment with an amino acid mixture or lactose. Rokumi-gan has beneficial amino acids/proteins, at least in part, contributing to keep levels of serum amino acids constant for several hours.

MATERIALS AND METHODS

Subjects

Six healthy men (age 35.5 ± 4.8 years, height 168 ± 3.5 cm, body weight 67.7 ± 4.8 kg, body mass index 23.9 ± 0.9 kg/m^2) participated in the experiment after giving informed consent to the experimental protocol, which had been approved by the ethical committee of The Komatsushima Hospital. All subjects had physical examinations performed by a physician before participating in this study.

Administration of Rokumi-gan, amino acid mixture and lactose

This study was designed as a lactose-amino acid mixture-Rokumi-gan randomized crossover trial. The following treatments were administered : lactose treatment, 5 g of lactose ; amino acid mixture treatment ; 5 tablets of amino GET (Asahi, Osaka, Japan) ; prescription treatment, 10 g of commercially prepared Rokumi-gan (TJ-87) purchased from Tsumura Pharmaceutical Co. (Tokyo, Japan). Since commercially prepared Rokumi-gan contained 5 g of lactose as an expicient, we used 5 g of lactose as control. Five tablets of amino Get contains almost similar amounts of Ala, Arg, Asp, Glu, Gly, Phe and Ser with those in 10 g of commercially prepared Rokumi-gan (Ala, 25.2 ; Arg, 49.8 ; Asp, 48.6 ; Cys, 3.2 ; Glu 99.6, Gly 19.0 ; Gln, 59 His 7.8 ; Ile, 77.6 ; Leu, 104 ; Lys 26.0 ; Met, 5.6, Phe, 13.8 ; Pro, 15.2, Ser, 18.4 ; Thr, 16.8 ; Trp, 4.2 ; Tyr, 9.6 ; Val, 71.2 mg/5 tablets), although Rokumi-gan did not contain cysteine, methionine, proline, threonine and tyrosine. A washout period of three month was maintained between the treatments. The subjects entered hospital one day before the each study day. From 21 : 00 on the day before each study no meal and beverage were allowed except for a free intake of water until 6 hrs after administration of a lactose, amino acid mixture or Rokumi-gan. During the same period, the subjects were allowed to be free, except for smoking and hard working. On the study day, a lactose, amino acid mixture or Rokumi-gan was administered to all subjects with 180 ml of water at 9 : 00. Blood samples were taken before or at 1, 2, 4, and 6 hr after the administration.

Determination of serum amino acid concentration

The blood samples were centrifuged for 10 min at 3,500 rpm to separate cells from serum, which was immediately frozen and stored at -80℃ until analysis. To measure serum amino acid concentration, the serum was subjected to amino acid analyzer, as described previously (9). Briefly, these samples were deproteinized with 5% sulphosalicylic acid and subjected to an automatic Hitachi L-8500 A amino acid analyzer (Hitachi, Tokyo, Japan) using cation exchange column with sulfonate group (2622-SC, 4.6 × 60 mm) and fluorescence detection. All samples were analyzed in duplicate.

Measurement of amino acid constitution in Rokumi-gan

We determined the amino acid constitution in two kinds of Rokumi-gan : commercially prepared drugs with three different lots (No. I, II and III) and self-decocted extract from the six plants. The plants were purchased from Tsumura Pharmaceutical Co. The production places of the original plants between commercially prepared (Lot No. I) and self-decocted drugs were same. Daily dose (7.5 g of dry powder) of the commercially prepared drug was made from 5 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Hoelen and 3 g of Moutan Bark. Therefore, we prepared the self-decocted extract from the same amounts of the six plants : the plants were incubated in 660 ml of water at 60℃ for 1 hr with a decoction apparatus (Uchida, Tokyo Japan). Aliquots of both samples were hydrolyzed with 6 N HCl at 121℃ for 16 hr. Following digestion, the samples were titrated to pH 4-5 with 5 M KOH and centrifuged to remove precipitate and were subjected to amino acid analysis as described above.

Statistical analysis

All data were expressed as mean ± SEM, n = 3 or 6. One-way ANOVA followed by Dunnett's test was used to determine the different amounts of amino acids between self-decocted and commercially-prepared Rokumi-gan, or to evaluate the significant effects of Rokumi-gan, amino acid mixture and lactose on serum amino acid concentration, compared with that before treatment with each agent. We also applied the results to the modified Bonferroni correction, Holm procedure, for multiple testing (10) to compare the distinct effects of Rokumi-gan, amino acid mixture and lactose on serum amino acid concentration at the indicated periods after the treatment. These analyses were performed using SPSS computer programs (SPSS Japan Inc., Tokyo).

RESULTS

Amino acid amounts in commercially prepared and Rokumi-gan with three different lots

Commercially prepared Rokumi-gans with three different lots contained similar kinds and amounts of amino acids (Table 1). Especially, the Rokumi-gan contained high amounts (more than 20 mg/10 g Rokumi-gan) of arginine, aspartate and glutamate. It had alanine, phenylalanine and serine at the moderate levels (10-20 mg/10 g Rokumi-gan), and glycine, histidine, isoleucine, leucine, lysine and valine at the low levels (less than 10 mg/10 g Rokumi-gan). Cysteine, methionine, proline, threonine and tryptophan were hardly detected in the commercially prepared Rokumi-gan. Interestingly, Rokumi-gan without hydrolyzation contained few amounts of amino acids, except for arginine (Table 1), indicating that almost of all amino acids were present as peptides or proteins. Unexpectedly, Rokumi-gan contained a high amount of free arginine.

Amino acid amounts in self-decocted Rokumi-gan

Self-decocted Rokumi-gan significantly contained 1.6-fold-higher amounts of total amino acids (Table 2). Amounts of glutamate, isoleucine and serine in self-decocted Rokumi-gan were significantly higher than those in commercially prepared drug, whereas self-decocted Rokumi-gan contained lower amounts

Table 1. Amino acid contents in Rokumi-gan with or without hydrolyzation.

Lot	No-hydrolyzation (mg/10 g Rokumi-gan)				Hydrolyzation (mg/10 g Rokumi-gan)				Fold
	I	II	III	Average	I	II	III	Average	
Ala	4.7 ± 0.5	7.1 ± 0.1	7.0 ±0.1	6.3	12.9 ± 1.4	16.8 ± 1.6	22.0 ± 3.5	17.2	2.7
Arg	45.8 ± 2.2	45.2 ± 0.7	42.8 ± 0.4	44.3	59.1 ± 1.7	58.8 ± 1.4	58.6 ± 1.1	58.8	1.3
Asp	2.8 ± 0.2	3.0 ± 0.1	2.7 ± 0.1	2.8	41.9 ± 4.0	41.0 ± 4.7	36.9 ± 6.7	39.9	14.3
Glu	1.2 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.4	55.5 ± 3.1	63.3 ± 3.0	60.5 ± 5.0	59.8	42.7
Gly	0.7 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0	13.5 ± 2.6	7.7 ± 0.1	8.4 ± 0.7	9.9	9.9
His	0	0	0	0	4.0 ± 0.4	3.6 ± 0.2	5.4 ± 1.0	4.3	
Ile	1.0 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	1.2	3.3 ± 0.5	3.8 ± 0.8	2.9 ± 0.2	3.3	2.8
Leu	1.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.2	1.0	6.4 ± 0.5	8.3 ± 0.8	7.4 ± 1.0	7.4	7.4
Lys	1.6 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	1.7	7.6 ± 0.1	8.4 ± 0.1	8.2 ± 0.1	8.1	4.8
Phe	8.2 ± 0.4	8.0 ± 0.8	9.0 ± 0.2	8.4	18.5 ± 0.5	17.9 ± 0.6	18.2 ± 0.4	18.2	2.2
Ser	5.3 ± 2.6	4.4 ± 0.1	4.5 ± 0.3	4.7	13.2 ± 0.6	16.2 ± 0.2	10.9 ± 2.3	13.4	2.9
Val	2.0 ± 0.1	1.9 ± 0.2	2.3 ± 0.1	2.1	5.8 ± 1.3	5.5 ± 0.2	4.2 ± 0.7	5.2	2.5
Total	74.5	76.1	74.9	74.9	231.7	251.3	243.6	245.4	3.3

We determined the amino acid constitution in commercially prepared Rokumi-gans with three different lots (No. I, II and III) before or after hydrolyzation. Hydrolyzation and amino acid analysis were performed as described in Materials and Methods. Cys, Met, Pro, Thr, Tyr, Asn, Gln and Trp were not detected in Rokumi-gan. However, Asn and Gln may be oxidized to Asp and Glu, respectively, in hydrolyzed Rokumi-gan, since the sample was treated with 6 N HCl.

Table 2. Amounts of amino acids in self-decocted Rokumi-gan.

	Self-decocted[1] (mg/10 g equivalent to commercially prepared)	Ratio to commercially prepared[1]
Ala	21.2 ± 0.4*	1.2
Arg	66.3 ± 0.8*	1.1
Asp	47.9 ± 0.4*	0.5
Glu	107.0 ± 0.4*	1.8
Gly	8.7 ± 0.4	0.9
His	3.6 ± 0.4	0.8
Ile	6.3 ± 0.4	1.9
Leu	7.9 ± 0.4	1.1
Lys	7.2 ± 0.4	0.9
Phe	9.1 ± 0.8	0.5
Ser	21.6 ± 0.4*	2.7
Val	9.6 ± 0.4	1.8
Cys, Met, Pro, Thr, Tyr	not detected	
Asn[2], Gln[2], Trp	not determined	
Total	316.4	1.6

[1]We hydrolyzed self-decocted Rokumi-gan with 6N HCl and determined the amounts of amino acids, as described in Materials and Methods. The ratio of amino acid contents of self-decocted to those of commercially prepared Rokumi-gan (See Table 1) was calculated.
[2]Asn and Gln were oxidized to Asp and Glu, respectively, since the sample was hydrolyzed with 6 N HCl.
Data are shown as mean ± SEM (n = 3). *Significantly different, compared with the value in commercially-prepared Rokumi-gan ($P < 0.05$).

of aspartate and phenylalanine than commercially prepared drug. There was no difference in amounts of other amino acids between self-decocted and commercially prepared drugs.

Changes in serum amino acid concentration after a single administration of Rokumi-gan

We measured serum amino acid concentrations in 6 healthy volunteers before or after administration of Rokumi-gan, an amino acid mixture or lactose. The actual value and relative ratio to the value before administration of serum acid concentration were shown in Table 3 and Fig. 1, respectively. In healthy volunteers treated with lactose, the concentrations of almost of all serum amino acids tested gradually decreased in time-dependent manner. Unexpectedly, treatment with an amino acid mixture containing similar amounts to Rokumi-gan hardly changed serum amounts of amino acids. Almost of all amino acids, except for arginine, were sustained at the basal level or gradually decreased after the treatment. In contrast, treatment with Rokumi-gan significantly increased the levels of serum alanine, serine and glycine which were contained in Rokumi-gan at the moderate or low levels, compared with treatment with an amino acid mixture. Rokumi-gan also increased the levels of serum arginine and glutamate, which were highly contained in Rokumi-gan. Almost of these amino acids showed peak values 1 or 2 hr after administration, whereas serum glutamate level gradually increased until 6 hr after administration. The increase in serum arginine level after treatment with Rokumi-gan was not statistically significant, compared with that after treatment with an amino acid mixture. This is consistent with the finding that Rokumi-gan contained a high amount of free arginine (Table 1). Rokumi-gan effectively prevented time-dependent declines in the amounts of serum histidine, isoleucine and leucine, which were observed in volunteers treated with lactose or an amino acid mixture. Serum aspartate was not detected in all samples, although there was aspartate at the high level in the powder.

Table 3. Changes in serum amino acid concentration after single administration of Rokumi-gan, amino acid mixture and lactose.

			Time after administration			
		0 hr (Before)	1 hr	2 hr	4 hr	6 hr
						(nmol/ml of serum)
Ala	Lactose :	389.6 ± 28.8	394.6 ± 31.5	382.6 ± 26.8	349.2 ± 25.8*	311.4 ± 20.9**
	Amino Acids :	335.7 ± 45.0	327.9 ± 39.0	310.6 ± 32.7	290.5 ± 24.2*	273.6 ± 24.2**
	Rokumi-gan :	256.3 ± 8.5	283.5 ± 11.4	293.4 ± 15.0*	262.5 ± 9.8	255.5 ± 14.9
Arg	Lactose :	75.7 ± 8.5	72.1 ± 8.0	72.8 ± 9.3	70.3 ± 8.3	72.8 ± 7.7
	Amino Acids :	81.5 ± 18.3	92.7 ± 13.0	81.1 ± 9.7	84.5 ± 10.9	81.0 ± 9.5
	Rokumi-gan :	78.9 ± 7.7	84.3 ± 8.1	86.5 ± 7.2	81.4 ± 6.4	77.7 ± 5.9
Glu	Lactose :	150.9 ± 18.6	152.3 ± 20.5	166.1 ± 18.1	162.5 ± 24.8	145.6 ± 19.8
	Amino Acids :	129.9 ± 27.8	113.3 ± 18.3	106.6 ± 18.7*	101.7 ± 18.3**	103.2 ± 20.0*
	Rokumi-gan :	164.2 ± 16.9	179.8 ± 15.3	178.8 ± 21.3	176.4 ± 20.6	183.0 ± 20.0
Gly	Lactose :	236.7 ± 17.0	235.3 ± 14.6	235.1 ± 18.8	224.7 ± 17.4	213.4 ± 18.4**
	Amino Acids :	252.7 ± 10.7	237.6 ± 13.6	233.0 ± 20.7	222.6 ± 18.8**	218.3 ± 18.7**
	Rokumi-gan :	256.3 ± 8.5	283.5 ± 11.4*	293.4 ± 15.0	262.5 ± 9.8	255.5 ± 14.9
His	Lactose :	88.6 ± 2.4	87.8 ± 2.5	86.5 ± 0.9	90.6 ± 2.6	89.6 ± 1.9
	Amino Acids :	89.2 ± 3.9	91.8 ± 4.2	87.2 ± 3.7	87.6 ± 2.4	85.3 ± 2.8
	Rokumi-gan :	94.3 ± 2.1	100.3 ± 2.8	99.6 ± 4.8	99.8 ± 5.5	100.3 ± 3.7
Ile	Lactose :	79.4 ± 4.0	72.7 ± 3.9*	69.5 ± 2.3**	69.3 ± 3.1**	71.2 ± 3.0**
	Amino Acids :	75.9 ± 3.0	77.6 ± 1.6	71.4 ± 2.0	64.9 ± 1.6**	63.1 ± 2.0**
	Rokumi-gan :	78.7 ± 4.9	77.4 ± 5.5	74.7 ± 4.2	75.1 ± 4.4	76.7 ± 3.6
Leu	Lactose :	148.9 ± 6.6	140.3 ± 7.0	139.1 ± 5.2	142.8 ± 5.8	147.0 ± 6.5
	Amino Acids :	157.9 ± 6.2	160.2 ± 5.7	152.4 ± 5.8	148.4 ± 5.0	146.6 ± 3.4*
	Rokumi-gan :	156.8 ± 8.7	158.3 ± 9.9	155.9 ± 9.9	160.3 ± 9.9	162.1 ± 7.5
Lys	Lactose :	205.9 ± 5.2	206.5 ± 5.7	211.3 ± 8.0	221.6 ± 11.5	221.6 ± 8.1
	Amino Acids :	224.5 ± 11.8	216.9 ± 10.3	209.5 ± 7.2	211.6 ± 7.1	206.6 ± 7.4
	Rokumi-gan :	241.5 ± 14.8	237.5 ± 13.9	243.7 ± 17.7	242.8 ± 16.3	243.2 ± 10.8
Phe	Lactose :	72.5 ± 1.8	70.1 ± 1.5	74.4 ± 2.0	73.4 ± 1.3	71.8 ± 1.2
	Amino Acids :	63.3 ± 9.1	59.7 ± 4.1	55.9 ± 4.9	55.0 ± 4.6	51.8 ± 5.0
	Rokumi-gan :	86.5 ± 4.0	84.8 ± 3.7	85.2 ± 7.1	82.0 ± 4.5	83.5 ± 4.3
Ser	Lactose :	124.4 ± 4.8	126.7 ± 4.6	132.3 ± 6.9	126.2 ± 3.9	123.5 ± 4.3
	Amino Acids :	141.5 ± 6.9	125.6 ± 9.6	118.4 ± 8.6*	121.1 ± 7.6	117.7 ± 7.7*
	Rokumi-gan :	135.5 ± 3.9	162.9 ± 9.1**	147.4 ± 8.1	143.2 ± 5.4	144.5 ± 2.9
Val	Lactose :	263.0 ± 8.6	253.6 ± 9.0	252.2 ± 7.3	249.4 ± 7.9	249.3 ± 7.8
	Amino Acids :	241.1 ± 9.6	245.4 ± 8.2	237.2 ± 8.8	233.3 ± 6.0	233.4 ± 6.0
	Rokumi-gan :	263.5 ± 17.1	271.2 ± 17.0	267.2 ± 20.9	264.0 ± 19.3	265.4 ± 14.0

Asn, Cys, Gln, Met, Pro, Thr, Trp, Tyr = not determined, Asp = not detected

Rokumi-gan (10 g) and lactose (5 g) were administered to the same 6 healthy volunteers with 180 ml of water at 9 : 00. A washout period of three month was maintained between both treatments. The volunteers were not allowed meal and beverage from 21 : 00 on the day before each study, except for a free intake of water. Blood samples were taken before or at 1, 2, 4, and 6 hr after the administration. Then, serum was subjected to amino acid analysis. Data are shown as mean ± SEM (n = 6). *, **Significantly different, compared with the value before treatment with each reagent ($P < 0.05$ and $P < 0.01$, respectively).

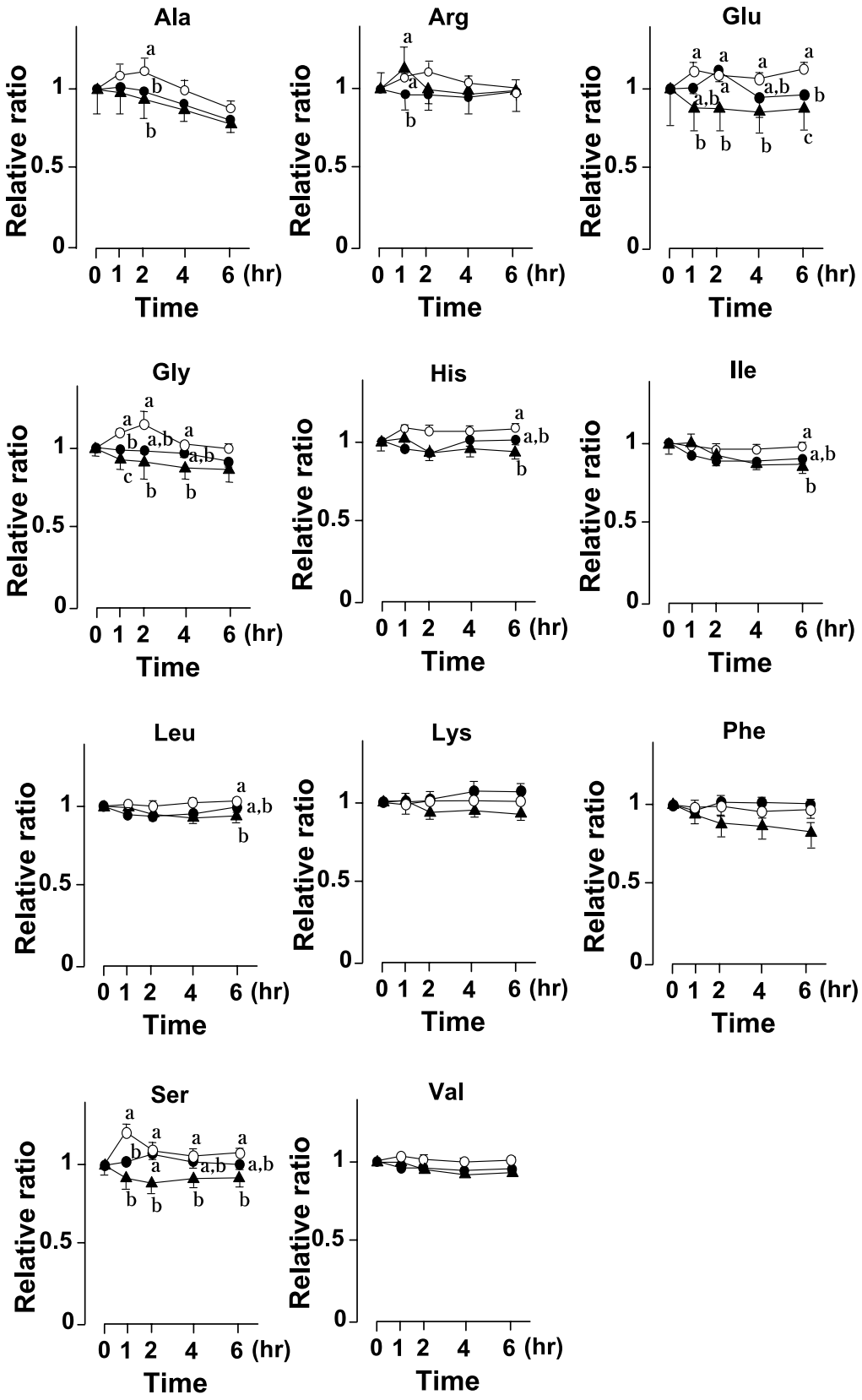

Fig. 1. Relative changes in serum amino acid concentration after single administration of Rokumi-gan.

Rokumi-gan (10 g ; —○—), amino acid mixture treatment (5 tablets of amino GET ; —▲—) and lactose (5 g ; —●—) were administered to the same 6 healthy volunteers with 180 ml of water at 9 : 00. A washout period of three month was maintained between both treatments. The volunteers were not allowed meal and beverage from 21 : 00 on the day before each study, except for a free intake of water. Blood samples were taken before or at 1, 2, 4, and 6 hr after the administration. Then, serum was subjected to amino acid analysis. Data are shown as the relative value to that before the treatment and mean ± SEM (n = 6). Values with different superscripts are significant within the values at the indicated periods after treatment with each reagent ($P < 0.05$).

DISCUSSION

This is the first report, to our knowledge, that Rokumi-gan (TJ-87) contained relative high amounts of various amino acids (4.9% in dried extracts excluding expicient), especially glutamate, arginine and aspartate, and that administration of Rokumi-gan could increase or sustain serum concentrations of several amino acids in human subjects even 6 hrs after treatment, compared with the treatment with an amino acid mixture or lactose. Based on these findings, Rokumi-gan may be a beneficial amino acid supplier for keeping serum concentration constant.

Rokumi-gan contained glutamate and arginine at the relative high levels. Recently, the evidence for beneficial effects of these amino acids has been accumulated. For example, oral supplementation with glutamate improved anorexia in rats (11). Dietary *L*-arginine supplementation improved the glomerular filtration rate and renal blood flow after 24 hours of unilateral ureteral obstruction in rats (7). Furthermore, arginine improved erectile dysfunction by increasing the amounts of endogenous nitric oxide and stimulated secretion of growth hormone (8, 12). Interestingly, Rokumi-gan is effective against anorexia, asthenia of kidney, vigor and slow growth of children (2, 13, 14), although the mechanism was not proven. Such amino acids in Rokumi-gan may contribute, at least in part, to its pharmaceutical effects.

Protein-restricted diets are more effective for renal dysfunction, such as glomerular nephritis, nephrosis and renal failure, because they decrease urogenesis leading to the decrease in blood urea nitrogen (BUN) (15). Daily protein intake in patients with renal failure was restricted up to 50% of that (50 - 70 g/day) in normal persons. In contrast, several amino acids themselves have beneficial effects on renal function, and relative low amounts (at most 1 g/day) of these amino acids are necessary for their pharmaceutical effects (16). Therefore, it is unlikely that amino acid supplementation with Rokumi-gan (less than 250 mg/daily dose of Rokumi-gan) causes the side-effects on renal function.

In subjects treated with a Rokumi-gan, serum concentrations of almost of all tested amino acids, except for glutamate, showed the peak values within 2 hrs after treatment. However, an amino acid mixture supplement containing similar amounts of amino acids in Rokumi-gan may be immediately absorbed and flushed out from blood within 1 hr after treatment, in consistent with the previous report that serum amino acids showed the peak value about 30 min after treatment with amino acid mixtures (17). There is the following reason why treatment with Rokumi-gan caused the distinct pattern of serum amino acid levels from those with an amino acid mixture. Amino acids in Rokumi-gan are present as peptides or proteins, because hydrolyzation increased amounts of amino acids in Rokumi-gan about 3 folds. Peptides or proteins in Rokumi-gan may be gradually digested after treatment, and Rokumi-gan could increase serum amino acid concentrations for a long time. However, we can not exclude another possibility that constituents in Rokumi-gan disturb the absorption of amino acids in small intestine. Further examinations are necessary to elucidate this possibility, since Rokumi-gan contains many unidentified constituents.

Comparison of amino acid constituents between pre-extracted drug and self-decocted extract revealed that self-decocted extract contained 1.6-fold higher amount of total amino acids than pre-extracted drug did. Especially, amounts of glutamate, isoleucine and serine in self-decocted extract were higher than those in pre-extracted drug. These amino acids may be destroyed during the preparation of pre-extracted drug. Based on these findings, self-decocted extract has bigger amounts of effectors than those in commercially prepared drug.

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