Effects of Ompi-to on renal anemia and platelet aggregation activity

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Abstract

In rats given an adenine diet, red blood cell count (RBC), hemoglobin level (Hb) and hematocrit (Ht) decreased gradually as the administration period was prolonged, providing evidence of renal anemia. Administration of Ompi-to improved renal anemia from day 18 in comparison with the control group. In addition, in rats given adenine with Ompi-to, platelet aggregation activity was suppressed on day 12, while that on day 24 had enhanced.

Key words Renal anemia, platelet aggregation, Ompi-to, adenine-fed rat.

Abbreviations Hb, hemoglobin level ; Ht, hematocrit ; MA, maximum aggregation rate ; MCH, mean corpuscular hemoglobin ; MCHC, mean corpuscular hemoglobin concentration ; MCV, mean corpuscular volume ; Ompi-to (Wen-Pi-Tang), 温脾湯 ; PLT, platelet count ; PPP, platelet-poor plasma ; PRP, platelet-rich plasma ; RBC, red blood cell count.

Introduction

Ompi-to is an oriental medical prescription which has been used empirically in China for the treatment of moderate chronic renal failure. In order to determine scientifically the effects of this drug, we previously administered Ompi-to to rats in combination with an adenine diet and found that Ompi-to produced a decrease in blood urea nitrogen and serum creatinine, and a marked decrease in methylguanidine and guanidinosuccinic acid accumulated in the body. It also led to improvement of hypalbuminotic hyperphosphatemia, partial improvement of blood hormone levels (decreased calcitonin, increased testosterone, improvement of the renin-angiotensin-aldosterone system, increased 3, 5, 3'-triiodothyronine and 3, 5, 3', 5'-tetraiodothyronine) and a decrease in blood pressure.[1-5] Thus, Ompi-to was proved to have a prophylactic effect on the progression of renal failure. In addition, in a model experiment for clinical application, we produced chronic progressive renal failure and investigated the effects of Ompi-to on this condition. It was found that chronic progression of renal failure was inhibited, suggesting the usefulness of Ompi-to for drug treatment of renal failure.[6] In chronic renal failure, anemia is one of the most frequent symptoms, leading to acquired platelet dysfunction.[7] In this connection, we carried out the present study to determine the effects of Ompi-to on renal anemia and platelet aggregation activity.

Materials and Methods

Animals and treatment : Male rats of the LWH : Wistar strain, with a body weight of about 200 g, were used. The rats were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 23°C)and humidity (about 60 %) were controlled automatically. The animals were fed on an 18 % casein diet containing 0.75 % adenine, which produced experimental renal failure. In rats with adenine - induced renal failure, renal impairment becomes aggravated as the period of adenine feeding is prolonged.[8-14] Ompi-to extract

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was dissolved in water, and given to rats orally every day as drinking water with the adenine diet. The dose was adjusted to 40 or 80 mg/day by regulating its concentration in relation to water consumption. Control rats were given a corresponding amount of water. Throughout the experiment there were no significant differences in body weight change between the control and extract-treated rats. The food intake of each rat was essentially proportional to weight change. Six rats were used for each experimental group. Values were expressed as mean±S.E.

Ompi-to : The Ompi-to preparation was the same as that described previously.[1] The composition of Ompi-to used in this experiment was as follows : 15 g of Rhei Rhizoma (*Rheum officinale* BAILLON), 3 g of Ginseng Radix (*Panax ginseng* C. A.MEYER), 5 g of Glycyrrhizae Radix (*Glycyrrhiza glabra* LINN. var. *glandulifera* REGEL et HERDER), 3 g of Zingiberis Rhizoma (*Zingiber officinale* ROSCOE) and 9 g of Aconiti Tuber (*Aconitum japonicum* THUNBERG). Ginseng Radix was a product of Korea, Aconiti Tuber was from Japan, and the other ingredients were from China.

Blood indices : Blood samples were collected from the heart without anesthesia, and the red blood cell count (RBC), hemoglobin level (Hb), hematocrit (Ht) and platelet count (PLT) in the blood were determined with a Coulter counter (T-540).

Platelet aggregation activity : A cannula was inserted into the carotid artery of each rat, and the blood was sampled with 3.13 % citric acid. Blood samples were centrifuged at 600 rpm for 10 min to obtain platelet-rich plasma (PRP). After separation of PRP, the samples were further centrifuged at 3,000 rpm for 10 min to obtain platelet-poor plasma (PPP). The PRP samples were diluted with PPP to obtain a platelet concentration of $50\times10^4/\mu l$. An aggregometer (NKK Hematracer I, Niko Bio-science) was used for determination of the platelet aggregation activity. ADP and collagen were used to induce aggregation. The aggregation activity with ADP was expressed as the concentration of ADP required for an aggregation rate of 50 % at double the time required to reach the maximum aggregation, and the aggregation activity with collagen was expressed as the maximum aggregation rate at the threshold dose and time until the beginning of aggregation (lag time=$T_{1/2max}$).

Statistics : The significance of differences between the normal rats and those with renal failure treated or non-treated with Ompi-to extract was tested using Student's t test. Differences at a p value of less than 0.05 were considered to be statistically significant.

Results

Blood indices

As shown in Table I, RBC, Hb and Ht in rats given adenine alone decreased along with the course of adenine administration, reaching values 41 %, 43 % and 44 % lower, respectively, than the corresponding values in normal rats on day 24. On the other hand, in rats given adenine with Ompi-to extract at 40 mg/day, RBC, Hb and Ht became significantly higher than those in control rats with renal failure on day 18, reaching values 14 %, 17 % and 16 % higher, respectively, than those on day 24. Further increase in the dose to 80 mg/day produced a further increase ; however, the value did not show a direct correlation with the amount of extract administered. Unlike RBC, Hb or Ht, there was an increase in PLT along with the progression of renal failure in rats given adenine alone, reaching a value 45 % higher than that in normal rats on day 24. In contrast, there were almost no changes in these indices in rats given Ompi-to at a dose of 40 mg/day or 80 mg/day.

Erythrocyte indices

Mean corpuscular volume (MCV, Ht/RBC×10), mean corpuscular hemoglobin (MCH, Hb/RBC×10) and mean corpuscular hemoglobin concentration (MCHC, Hb/Ht×100) all decreased along with the course of adenine administration ; on day 24, MCV and MCH were significantly lower, by 11 % and 15 %, respectively, in rats given adenine than in normal rats, whereas MCHC in the former was only slightly higher than that in the latter (Table II). In rats with renal failure given Ompi-to 40 mg/day or 80 mg/day, the values of MCV, MCH and MCHC were almost

Table I Effects of Ompi-to extract on blood indices.

Day	Group	Dose (mg/day)	RBC ($\times10^6$/mm^3)	Hb (g/dl)	Ht (%)	PLT ($\times10^4$/mm^3)
6	Rats with renal failure					
	Control	−	10.15±0.18	18.42±0.23	56.81±1.16	111.4±6.0^b
	Ompi-to extract	40	9.63±0.32	17.91±0.49	55.09±1.63	110.0±5.7^b
	Ompi-to extract	80	10.13±0.23	18.37±0.30	56.88±1.16	112.1±3.6^c
12	Rats with renal failure					
	Control	−	8.46±0.12^c	15.16±0.25^c	46.28±0.76^c	119.2±7.3^b
	Ompi-to extract	40	8.63±0.24^c	15.66±0.44^c	48.33±1.26^c	123.6±6.4^c
	Ompi to extract	80	8.96±0.36^b	16.16±0.65^b	49.73±1.99^{b,d}	128.9±6.5^c
18	Rats with renal failure					
	Control	−	6.85±0.23^c	11.85±0.54^c	36.80±1.29^c	124.7±9.3^b
	Ompi to extract	40	7.53±0.22^{c,d}	13.55±0.38^{c,d}	42.28±1.23^{c,d}	125.3±6.5^c
	Ompi-to extract	80	7.81±0.25^{c,d}	14.02±0.49^{c,d}	42.56±1.44^{c,d}	131.4±4.2^c
24	Rats with renal failure					
	Control	−	6.20±0.37^c	10.63±0.65^c	33.03±2.02^c	129.2±11.2^b
	Ompi-to extract	40	7.09±0.20^{c,d}	12.46±0.44^{c,d}	38.32±1.41^{c,d}	133.0±7.1^c
	Ompi-to extract	80	7.44±0.30^{c,d}	13.14±0.59^{c,d}	39.04±1.22^{c,d}	135.8±4.5^c
	Normal rats		10.57±0.17	18.61±0.18	59.18±1.10	89.1±1.1

RBC, red blood cell count; Hb, hemoglobin level; Ht, hematocrit; PLT, platelet count. Statistical significance : $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* normal rats, $^d p<0.05$ *vs.* control rats with renal failure.

Table II Effects of Ompi-to extract on erythrocyte indices.

Day	Group	Dose (mg/day)	MCV (μm^3)	MCH (pg)	MCHC (%)
6	Rats with renal failure				
	Control	−	55.93±0.28^b	18.17±0.19^b	32.49±0.40
	Ompi-to extract	40	56.55±0.35^a	18.43±0.15^b	32.53±0.15^a
	Ompi-to extract	80	56.16±0.41^b	18.14±0.21^b	32.30±0.18^b
12	Rats with renal failure				
	Control	−	54.70±0.26^c	17.92±0.17^c	32.76±0.29
	Ompi-to extract	40	56.01±0.89^a	18.14±0.13^c	32.38±0.13^a
	Ompi-to extract	80	55.59±0.47^b	18.04±0.22^b	32.48±0.18^a
18	Rats with renal failure				
	Control	−	53.32±0.29^c	17.16±0.36^c	32.15±0.48^a
	Ompi to extract	40	55.98±0.64^{b,e}	18.03±0.14^{c,d}	32.37±0.35^a
	Ompi-to extract	80	56.74±0.57^{a,f}	18.09±0.20^{b,d}	32.11±0.17^b
24	Rats with renal failure				
	Control	−	53.07±0.20^c	17.09±0.10^c	32.19±0.12^b
	Ompi-to extract	40	54.31±0.21^{c,e}	17.67±0.11^{c,e}	32.52±0.16^a
	Ompi-to extract	80	55.05±0.41^{b,e}	17.85±0.19^{c,e}	32.43±0.34^a
	Normal rats		59.61±0.92	20.03±0.39	33.56±0.35

MCV, mean corpuscular volume ; MCH, mean corpuscular hemoglobin ; MCHC, mean corpuscular hemoglobin concentration. Statistical significance : $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* normal rats, $^d p<0.05$, $^e p<0.01$, $^f p<0.001$ *vs.* control rats with renal failure.

the same as those in the controls on days 6 and 12. However, MCV and MCH on days 18 and 24 were significantly higher than the control values. MCHC did not show such an increase on day 18 or 24.

Platelet aggregation activity

ADP - induced aggregation: As shown in Table III, platelets in rats on day 12 of adenine administration became aggregated at an ADP concentration about 20 % lower than the corresponding concentration in normal rats. However, on day 24, the concentration required for aggregation was 4.44-fold higher than that in normal rats. The aggregation activity in rats given Ompi-to extract at 40 mg/day for 12 days was similar to that in control rats with renal failure, whereas it was significantly (72 %) lower in rats given Ompi-to at 80 mg/day for 12 days than in the controls. On day 24, the aggregation activity in control rats with renal failure was about 5.58-fold lower than that on day 12. The corresponding activity in rats given Ompi-to at 40 mg/day was higher, and that in rats given 80 mg/day was further higher (by 64 %) in comparison with the controls.

Table III Effects of Ompi-to extract on ADP-induced platelet aggregation.

Day	Group	Dose (mg/day)	C_{50} (μM)
12	Rats with renal failure		
	Control	–	13.73±1.82
	Ompi-to extract	40	14.90±1.22
	Ompi-to extract	80	23.67±2.19[d]
24	Rats with renal failure		
	Control	–	76.67±1.67[b]
	Ompi-to extract	40	45.92±11.44[a,c]
	Ompi-to extract	80	27.67±5.78[c]
	Normal rats		17.26±2.35

C_{50}, concentration of an inducer on 50% aggregation. Statistical significance: [a]$p<0.05$, [b]$p<0.001$ *vs.* normal rats, [c]$p<0.05$, [d]$p<0.01$, [e]$p<0.001$ *vs.* control rats with renal failure.

Collagen-induced aggregation : In rats given adenine for 12 days, the maximum aggregation rate (MA) was about 7 % higher, and the lag time about 42 % lower, than those in normal rats, showing slightly accelerated aggregation. However, on day 24, the MA was about 20 % lower, and the lag time about 30 % higher, than in normal rats, showing decreased aggregation activity in contrast with the state on day 12 (Table IV). In rats with renal failure given Ompi-to at 40 mg/day for 12 days, the MA was decreased significantly by 16 %, and the lag time increased about 2.21-fold. In rats given a dose of 80 mg/day, the aggregation activity was also decreased, though less conspicuously than in rats given 40 mg/day. After 24 days of Ompi-to administration at 40 mg/day or 80 mg/day, the MA was increased significantly by 19 % and 20 %, respectively, and the lag time was decreased by 29 % and 26 %, respectively, being restored to near-normal levels.

Table IV Effects of Ompi-to extract on collagen-induced platelet aggregation.

Day	Group	Dose (mg/day)	MA (%)	Lag time (min)
12	Rats with renal failure			
	Control	–	76.68±1.67	0.76±0.05
	Ompi-to extract	40	64.25±5.02[b]	1.68±0.32[b]
	Ompi-to extract	80	70.00±5.25	1.15±0.12[b]
24	Rats with renal failure			
	Control	–	57.75±3.04[a]	1.71±0.16
	Ompi-to extract	40	68.72±2.29[b]	1.22±0.13[b]
	Ompi-to extract	80	69.46±3.04[b]	1.26±0.15
	Normal rats		71.80±1.66	1.32±0.31

M.A., maximum aggregation rate. Statistical significance : [a]$p<0.01$ *vs.*normal rats, [b]$p<0.05$ *vs.* control rats with renal failure.

Discussion

Erythropoietin has frequently been used for the treatment of renal anemia, and its efficacy on anemia has been documented by many researchers. However, it produces a serious problem, hypertension, as a side effect. Raine and Roger [15] found an increase in blood pressure in about 1/3 of their patients with end-stage renal failure, and pointed out the involvement of blood viscosity and reversal hypoxic vasodilation in this phenomenon, on the basis of the fact that systemic vascular resistance was increased in all patients.

The present test drug, Ompi-to, is an oriental medical prescription containing the active compo-

nent rhubarb. This prescription was prescribed in "Bei Ji Qian Jin Yao Fang" published during the Tang period in China, and has been used empirically in Chinese medicine for the treatment of moderate chronic renal failure. We have previously carried out scientific studies of this prescription and found that it improves uremic conditions and renal function, decreases blood pressure and increases blood flow in renal and cerebral tissues.[1,6,16-18] A decrease in vascular resistance after administration of the drug has also been suggested. Since these effects were also present after prolonged administration, we consider that Ompi-to can eliminate or reduce the side effects of erythropoietin.

On the other hand, in rats given adenine alone in the present study, RBC, Hb and Ht decreased gradually along with the course of administration, providing evidence of renal anemia. In contrast, in rats given adenine and Ompi-to orally every day, RBC, Hb and Ht began to increase significantly on day 18 in comparison with the control group, reflecting the action of Ompi-to in improving renal anemia.

Relative deficiency of erythropoietin, suppressed myelopoiesis due to uremic toxins and accelerated erythrocyte destruction (shortened erythrocyte life span) have been cited as the major causes of renal anemia.[19] We have found previously that Ompi-to removes uremic toxins.[1,2,4,6] In the present study, although erythropoietin was not examined, MCV and MCH were found to increase significantly after 18 and 24 days of Ompi-to administration at 40 mg/day or 80 mg/day. These findings suggest enhanced hematopoiesis, i.e., improvement of renal anemia.

Renal failure is a pathological condition known to be associated with platelet dysfunction and is assessed in terms of platelet count or function.[20] In the present experimental model, the platelet count tended to increase, and there were no significant variations in this parameter after Ompi-to administration. In the presence of either ADP or collagen as an inducer, platelet aggregation activity was slightly increased on day 12 of adenine administration, and there was a decreased aggregation activity on day 24. However, in rats given adenine with Ompi-to, aggregation was suppressed on day 12, while that on day 24 had enhanced; in particular, the aggregation activity was restored to a near-normal level in rats given a dose of 80 mg/day.

Although the aspect of platelet function which is disturbed by uremia and improved by Ompi-to remains to be elucidated, it has been reported by Remuzzi *et al.*,[21] Turney *et al.*[22] and Castillo *et al.*[23] that disturbances in platelet function include the binding of factor V and phospholipids to the platelet membrane, impaired clot retraction by platelets, disturbed binding between platelets via fibrinogen, and decreased or increased activity of cyclooxygenase necessary for thromboxane A_2 production via the metabolism of arachidonic acid in platelets. On the other hand, Nenci *et al.*[24] have reported that bleeding and prolonged bleeding time in patients with renal failure were improved by hemodialysis, and pointed out the involvement of uremic toxins accumulated in the blood, such as urea, guanidinosuccinic acid and phenol. As reported previously,[1,2,4,6] various uremic toxins in the blood are markedly and significantly decreased after Ompi-to administration. Therefore, we consider that these uremic toxins are involved in the improvement of platelet function after Ompi-to administration.

The findings of the present study, when considered together with previous findings including decreased blood pressure, increased tissue blood flow and decreased vascular resistance, suggest that Ompi-to is also a therapeutic agent for renal anemia, but not of the erythropoietin type. Beneficial effects of this prescription on the aggregation, fibrinolysis and platelet systems are also expected. Thus, the old oriental medical prescription Ompi-to is also a contemporary drug.

和文抄録

アデニン投与ラットのRBC数，Hb量，Ht値は投与日数の経過とともに次第に低下し，腎性貧血を呈していた。しかしアデニン投与と同時に温脾湯を連日経口投与したラットでは投与18日目からRBC数，Hb量，Ht値がいずれも有意に増加し，腎性貧血を是正する作用が認められた。また12日目では

血小板凝集の抑制，24日目では逆に促進作用が温脾湯投与群で認められた。

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