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Effects of crude drug extract of Ompi-to on renal function in rats with renal failure

Takako YOKOZAWA,* Xiao Qi WU, Koji FUJIOKA and Hikokichi OURA

Department of Applied Biochemistry, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University

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Abstract

The effects of each crude drug extract of Ompi-to on renal function were investigated in rats with adenine-induced renal failure, and the following results were obtained. (1) The glomerular filtration rate was increased significantly by administration of Rhei Rhizoma or Aconiti Tuber. Ginseng Radix also caused a significant increase or a tendency toward an increase. In contrast, there was a tendency toward a decrease in rats given Glycyrrhizae Radix. (2) The renal plasma flow was increased significantly by administration of Rhei Rhizoma, Aconiti Tuber or Ginseng Radix. (3) The renal blood flow showed variations similar to those in renal plasma flow, being increased significantly by administration of Rhei Rhizoma, Aconiti Tuber or Ginseng Radix. However, unlike the former three crude drugs, administration of Glycyrrhizae Radix or Zingiberis Rhizoma caused a tendency toward a decrease in renal blood flow.

Key words Ompi-to (Onpi-tô), Rhei Rhizoma, Ginseng Radix, Aconiti Tuber, glomerular filtration rate, renal plasma flow, renal blood flow.

Abbreviation Ompi-to (Wen-Pi-Tang), 温脾湯.

Introduction

Each prescription in traditional Chinese medicine is composed of various crude drugs, which are used on the basis of the symptoms and signs employed in oriental diagnosis. The therapeutic effects of each prescription are achieved through wide-ranging and complex actions. The authors have been carrying out studies on rats with experimental renal failure in order to analyze these Chinese prescriptions, investigate the physiologically and pharmacologically active substances contained in their component crude drugs, and elucidate the action mechanisms of these substances with the aim of applying them clinically.[1,2] One of our findings is that Ompi-to, which is currently used on a practical basis in China for moderate renal failure, reduces the accumulation of uremic toxins in the body and facilitates their excretion through strong activation of renal function.[3-5] Ompi-to is composed of 5 kinds of crude drugs. In the present study, we investigated the extent to which each crude drug contributes to the therapeutic effect of Ompi-to, using various parameters of renal function.

Materials and Methods

Animals and treatment: Male rats of the Wistar strain, with a body weight of 200－210 g, were placed in metabolic cages and kept at a temperature of 23±1℃ under a 12-hr 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,

*〒930-01 富山市杉谷 2630
富山医科薬科大学和漢薬研究所臨床利用部門　横澤隆子
Sugitani, Toyama 930-01, Japan

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which produced experimental renal failure in the animals.[6-12] During the adenine-feeding period, an aqueous solution of each crude drug extract of Ompi-to was administered orally for 24 days as drinking water, while control rats received tap-water. On the 24th day of the experimental period, renal function tests were performed. Throughout the experimental period, there were no statistically significant differences between the controls and the rats treated with each crude drug extract of Ompi-to with regard to changes in body weight. The food intake of each rat was essentially proportional to weight change. In addition, daily intake of drinking water showed no appreciable changes in each group. No case of diarrheal symptoms was found. The blood urea nitrogen values of the rats used in this experiment reached an abnormally high value of about 120 mg/dl on the 24th experimental day. The creatinine values were also significantly increased to 4.2 times (3.82±0.21 mg/dl) those of normal rats. Six to seven rats were used for each experimental group. Values were expressed as means ± S.E.

Crude drugs : The crude drugs used in this experiment were as follows : Rhei Rhizoma (*Rheum officinale* BAILLON), Ginseng Radix (*Panax ginseng* C.A. MEYER), Glycyrrhizae Radix (*Glycyrrhiza glabra* LINN. var. *glandulifera* REGEL et HERDER), Zingiberis Rhizoma (*Zingiber officinale* ROSCOE) and Aconiti Tuber (*Aconitum japonicum* THUNBERG). Ginseng Radix was a product of Korea, Aconiti Tuber was from Japan and the other ingredients were from China. One hundred grams of each crude drug was boiled gently in 1000 ml of water for 5—65 min, according to the Ompi-to preparation procedure described previously.[3] The extract was then concentrated under reduced pressure to leave a residue. The yields of Rhei Rhizoma, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma and Aconiti Tuber were 21%, 32%, 20%, 11% and 37%, respectively. In the present study, doses of 20 and 40 mg/rat/day were used for Rhei Rhizoma, and 10 and 20 mg/rat/day for Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma and Aconiti Tuber. In comparison with the proportions of these crude drugs in the Ompi-to prescription, these doses excluding those of Rhei Rhizoma and Aconiti Tuber, *i.e.*, those of Ginseng Radix, Glycyrrhizae Radix and Zingiberis Rhizoma, are about twice or three times higher.

Examination of renal function : The glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by renal clearance test using a single intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as an indicator.[13,14] At 25 min after intravenous administration of either of these agents, the bladder was reflexly emptied by having each rat inhale ether for 3—5 sec. The urine thus voided was discarded. During the next 30 min, the urine was collected, and collection was terminated after the bladder had again been emptied reflexly by ether inhalation. Blood samples were taken from conscious rats by heart puncture in the middle of the period used for the clearance test. Thiosulfate and *para*-aminohippurate were determined by titrimetry and colorimetry, respectively. Renal blood flow (RBF) was calculated on the basis of RPF and hematocrit (Ht) using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

$$RBF = \frac{RPF}{1-Ht} \text{ (ml/min)}$$

Statistics : The significance of differences between the control and crude drug-treated groups was tested using Student's *t* test.

Results

Rhei Rhizoma : Table I shows the effect of Rhei Rhizoma on renal function. The GFR in adenine-administered control rats was maintained within the range of 0.54±0.10 ml/min/kg, whereas in rats following Rhei Rhizoma administration, there was a 70% increase in the 20-mg group and an 81% increase in the 40-mg group, both increases being statistically significant. The RPF value was 128% and 177% higher in rats given 20 mg and 40 mg, respectively, than in control rats, showing a greater effect of Rhei

Table I Effect of Rhei Rhizoma on renal function parameters.

Group	Dose (mg/rat/day)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	0.54±0.10 (100)	1.59±0.39 (100)	2.57±0.66 (100)
Rhei Rhizoma	20	0.92±0.05 (170)**	3.63±0.34 (228)**	6.10±0.56 (237)**
Rhei Rhizoma	40	0.98±0.12 (181)*	4.41±0.78 (277)*	7.55±1.35 (294)*

GFR, glomerular filtration rate ; RPF, renal plasma flow ; RBF, renal blood flow. Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$, **$p<0.01$.

Table II Effect of Ginseng Radix on renal function parameters.

Group	Dose (mg/rat/day)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	0.46±0.06 (100)	1.28±0.18 (100)	2.01±0.31 (100)
Ginseng Radix	10	0.60±0.19 (130)	2.34±0.41 (183)*	3.68±0.69 (183)*
Ginseng Radix	20	0.71±0.08 (154)*	2.26±0.24 (177)**	3.59±0.36 (179)**

Details are the same as in the legend to Table I. *Significantly different from the control value, $p<0.05$, **$p<0.01$.

Table III Effect of Glycyrrhizae Radix on renal function parameters.

Group	Dose (mg/rat/day)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	0.49±0.07 (100)	1.27±0.13 (100)	2.22±0.38 (100)
Glycyrrhizae Radix	10	0.39±0.04 (80)	1.14±0.17 (90)	2.11±0.36 (95)
Glycyrrhizae Radix	20	0.34±0.04 (69)	1.27±0.12 (100)	2.06±0.18 (93)

Details are the same as in the legend to Table I.

Rhizoma. Since there were no particular variations in the Ht value after Rhei Rhizoma administration, the RBF value calculated from RPF and Ht showed a variation pattern similar to that of the RPF value, *i.e.*, the RBF value was significantly increased by 137% and 194%, respectively, at 24 days in rats given 20 and 40 mg of the crude drug.

Ginseng Radix : Although the GFR value increased slightly by 30% at 24 days in rats given 10 mg of Ginseng Radix, administration of 20 mg Ginseng Radix significantly increased the GFR value from 0.46 ml/min/kg to 0.71 ml/min/kg (a 54% change, $p<0.05$), as shown in Table II. The RPF value in rats given 10 and 20 mg significantly increased by 83% and 77% of the level in control rats, respectively. The changes in RBF were almost the same as the changes in the RPF value.

Glycyrrhizae Radix : Table III shows the effect of Glycyrrhizae Radix on parameters of renal function after administration of oral doses of 10 and 20 mg/rat/day. The GFR value tended to be decreased in rats given either dose. There were no significant differences in the RPF and RBF values between the control and Glycyrrhizae Radix-treated groups, at either the 10 mg or 20

Table Ⅳ Effect of Zingiberis Rhizoma on renal function parameters.

Group	Dose (mg/rat/day)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	0.38±0.06 (100)	1.34±0.25 (100)	2.32±0.44 (100)
Zingiberis Rhizoma	10	0.63±0.04 (166)	2.18±0.46 (163)	3.61±0.57 (156)
Zingiberis Rhizoma	20	0.37±0.09 (97)	1.10±0.16 (82)	2.06±0.63 (89)

Details are the same as in the legend to Table Ⅰ.

Table Ⅴ Effect of Aconiti Tuber on renal function parameters.

Group	Dose (mg/rat/day)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	0.42±0.06 (100)	1.29±0.32 (100)	2.11±0.49 (100)
Aconiti Tuber	10	0.63±0.07 (150)*	2.49±0.38 (193)*	3.93±0.59 (186)*
Aconiti Tuber	20	0.72±0.09 (171)*	3.65±0.73 (283)*	5.88±1.19 (279)*

Details are the same as in the legend to Table Ⅰ. *Significantly different from the control value, $p<0.05$.

mg dosage level.

Zingiberis Rhizoma : As seen in Table Ⅳ, the GFR value showed a considerable (but not significant) increase in rats given 10 mg of Zingiberis Rhizoma, whereas further increase in the dose to 20 mg produced no differences in GFR between the control and Zingiberis Rhizoma-treated groups. Both the RPF and RBF values in rats given 10 mg also showed a considerable increase, whereas in contrast a tendency toward a decrease in both RPF and RBF was found in rats given the 20-mg dose.

Aconiti Tuber : As shown in Table Ⅴ, the GFR was 0.42 ml/min/kg in controls, whereas the values were significantly higher by 50% and 71% in rats given 10 mg and 20 mg of Aconiti Tuber, respectively. The RPF values were also markedly and significantly increased in rats given this crude drug, the value being 93% higher in the 10-mg group and 183% higher in the 20-mg group in comparison with the control group. In rats given 10 mg and 20 mg of Aconiti Tuber, the RBF values were 3.93 and 5.88 ml/min/kg, respectively (2.11 ml/min/kg in control rats).

Discussion

In chronic renal failure, various clinical symptoms, generically called uremia, develop along with deterioration of renal function. At this time, excretion of final metabolites from the kidneys is reduced because of the decreased renal function, resulting in accumulation of uremic toxins in the body.[15] The authors previously demonstrated that Ompi-to could be used as a new agent for conservative therapy, delaying the progression of renal failure by an action mechanism different from those of conventional low-protein, high-calorie therapy, essential amino acid therapy, and administration of activated charcoal and lactulose.[16] In the present study, in order to analyze the efficacy of Ompi-to, the effects of each of its crude drug extracts on renal function were investigated.

The results showed that a significant effect on GFR was achieved in rats given Rhei Rhizoma, Aconiti Tuber and Ginseng Radix. Rhei Rhizoma produced the strongest effect ; despite the decrease in GFR due to renal failure, activation

of renal function was noted in both the 20- and 40-mg groups. Aconiti Tuber was also an effective crude drug. After 24 days of administration, the GFR value was increased significantly by 50% and 71% in rats given 10 mg and 20 mg, respectively. The GFR tended to be increased in rats given 10 mg of Ginseng Radix, and was significantly increased in those given 20 mg. In contrast, there were almost no changes in rats given 20 mg of Zingiberis Rhizoma, and the value was decreased after administration of Glycyrrhizae Radix. A previous study on the effect of Ompi-to administration on GFR revealed that significant increases of 194% and 289% occurred in rats given 40 mg and 80 mg of Ompi-to, respectively, for 24 days.[5] As is clear from the results of the present study, Rhei Rhizoma, Aconiti Tuber and Ginseng Radix alone each caused a significant increase in GFR. The marked increase in GFR after Ompi-to administration can thus be explained by the additive action of these crude drugs.

On the other hand, a significant increase in RPF was noted in rats given Rhei Rhizoma, Aconiti Tuber or Ginseng Radix. As for Glycyrrhizae Radix, a tendency toward a decrease was noted in rats given 10 mg. A considerable increase was noted in rats given 10 mg of Zingiberis Rhizoma and a tendency toward a decrease occurred in rats given 20 mg. Thus, an evident effect on RPF was produced by Rhei Rhizoma, Aconiti Tuber and Ginseng Radix. On the basis of these findings, the effect of Ompi-to at 24 days is speculated to be attributable to a complex effect of Rhei Rhizoma, Aconiti Tuber and Ginseng Radix, rather than the action of only one of these crude drugs, as was suggested previously. Similar to the effects of Ompi-to on RPF, those on RBF at 24 days seem to be derived from a complex action of Rhei Rhizoma, Aconiti Tuber and Ginseng Radix.

Thus, an examination of the five individual crude drugs of Ompi-to in rats with renal failure revealed that even Rhei Rhizoma, Aconiti Tuber and Ginseng Radix, the three effective crude drugs, had a weaker effect on renal function in comparison with the corresponding effect of Ompi-to as a whole. Of these crude drugs, we have found that Rhei Rhizoma has a nitrogen metabolism-improving action.[17] Aconiti Tuber has long been known for its cardiotonic action, facilitating systemic circulatory efficiency and improving cardiovascular function.[18] Ginseng Radix has been reported to improve carbohydrate and lipid metabolism, facilitate the biosynthesis of DNA, RNA and protein, and have anti-anemic, cardiotonic, peripheral vasodilative and metabolism-activating actions.[19] It has also been reported that Zingiberis Rhizoma, which produced aggravation of renal function in the present study, facilitates blood circulation.[20] Therefore, it is considered that these crude drugs play a role in activating or normalizing the mechanism of homeostasis maintenance and exert a synergistic effect, thus improving renal function. It seems that further analysis of Ompi-to from other aspects would be useful, in view of the fact that its crude drugs have conflicting characteristics, *i.e.*, Rhei Rhizoma is a strong cold drug and Aconiti Tuber a strong warm drug.

和文抄録

温脾湯の各種構成和漢生薬エキスの腎機能に及ぼす影響をアデニン誘発腎不全ラットを用いて検討し，以下の結果が得られた。(1)糸球体濾過値は大黄，附子投与群において有意な増加作用を示し，人参投与群も有意な増加あるいは増加傾向を示した。これに対し，甘草投与群では逆に低下傾向を示した。(2)腎血漿流量は大黄，附子，人参投与群で有意に増加した。(3)腎血流量は大黄，附子，人参投与群で有意に増加したが，甘草，乾姜投与群では低下傾向を示した。

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