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Effects of Onpi-tô extract on renal function in rats with renal failure

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Abstract

Onpi-tô extract was administered to rats with renal failure induced by an adenine diet, and its effects on renal function parameters were investigated. Onpi-tô (5 mg or 10 mg/100 g body weight) caused a significant increase in renal plasma flow (RPF) and renal blood flow (RBF) in rats given adenine for 6 or 12 days, demonstrating increased renal function. In contrast, no such effect was found in rats after 18 or 24 days of adenine ingestion. Onpi-tô (5 mg or 10 mg/100 g body weight) tended to increase the glomerular filtration rate in rats given adenine for 6 or 12 days, whereas there was no such increase in rats given adenine for 18 or 24 days, similar to the results obtained for RPF and RBF.

Key words Onpi-tô, renal failure, renal function, renal plasma flow, renal blood flow, rat.

Abbreviation Onpi-tô (Wen-Pi-Tang), 温脾湯.

Introduction

We have so far investigated the effects of oriental drugs and prescriptions on experimental rats with renal failure, as a part of a research project on pharmacotherapy for chronic renal failure. Through such investigations, we have demonstrated that Onpi-tô, a preparation currently used experientially in Chinese medicine for the treatment of moderate renal failure, improves metabolism under conditions of renal failure, and have also reported data on its complex effects, thus elucidating the mechanism of its efficacy.[1-5] The renal function-improving action of the prescription has also been suggested by evidence of increased blood flow in the renal tissue, decreased blood pressure, partial suppression of the renin-angiotensin-aldosterone system and increased prostaglandin E levels.[6] In the present study, the effects of Onpi-tô extract on glomerular filtration rate, renal plasma flow and renal blood flow in rats with renal failure were examined, in order to further clarify its mode of action.

Materials and Methods

Animals and treatment : Male rats of the STD : 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, which produced experimental renal failure in the animals. In rats with renal failure induced by adenine, renal impairment becomes aggravated as the period of adenine feeding increases. It was previously confirmed by histological and biochemical procedures that renal failure was present after 6 days of ingestion.[7-10] Administration of the adenine diet to the rats for 6, 12, 18 or 24

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days was followed by intraperitoneal administration of Onpi-tô (5 mg or 10 mg/100 g body weight) dissolved in saline. Control rats were treated with an equal volume of saline. The blood urea nitrogen values of the rats used in this experiment reached a significantly increased level of 2.7 times (46.1±3.5 mg/dl) those of normal rats on the 6th experimental day and 3.5 times (59.7±3.9 mg/dl) those levels on the 12th experimental day. An abnormally high value of about 105 mg/dl was noted on day 24. Six rats were used for each experimental group. Values were expressed as means±S.E.

Onpi-tô : The Onpi-tô preparation was the same as that previously described.[1] The composition of Onpi-tô used in the 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. The extract was obtained by gently boiling the above-mentioned crude drugs 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 brown residue at a yield of about 20%.

Examination of renal function : Glomerular filtration rate (GFR), renal plasma flow (RPF), hematocrit value (Ht) and renal blood flow (RBF) values were obtained at 5.5—6.0 hr after intraperitoneal administration of the Onpi-tô extract, since the levels of urea and creatinine excretion were increased about 6 hr after Onpi-tô administration in rats given an adenine diet for 6 days. GFR and RPF were measured by means of renal clearance test using a single intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as an indicator.[11,12] At 25 min after intravenous administration of sodium thiosulfate or sodium *para*-aminohippurate, 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 bladders 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. RBF was calculated on the basis of RPF and Ht using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

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

Statistics: The significance of differences between the control and Onpi-tô extract-treated groups was tested by the use of Student's t test. A p value greater than 0.05 was considered to be statistically insignificant.

Results

The rats of the Onpi-tô extract-treated group showed a moderate increase in the GFR ; as shown in Table I, the GFR value on day 6 was 27% higher at an Onpi-tô extract dosage level of 5 mg/100 g body weight as compared with the control group, but this was not statistically significant. The GFR value was also increased by 41% as compared with the control upon intraperi-

Table I Effect of Onpi-tô extract on glomerular filtration rate.

Day	Material	Dose (mg/100 g B.W.)	GFR (ml/min/kg)
6	Control	-	2.11±0.39
	Onpi-tô	5	2.69±0.24
	Onpi-tô	10	2.97±0.43
12	Control	-	1.54±0.36
	Onpi-tô	5	1.88±0.65
	Onpi-tô	10	2.34±0.38
18	Control	-	1.21±0.14
	Onpi-tô	5	1.23±0.17
	Onpi-tô	10	1.15±0.05
24	Control	-	0.98±0.24
	Onpi-tô	5	1.07±0.25
	Onpi-tô	10	0.92±0.06

toneal administration of 10 mg / 100 g body weight (not significant). Similar changes produced by Onpi-tô administration were observed on day 12, even though renal impairment had increased due to extended administration of adenine. However, the GFR remained nearly unchanged after the administration of Onpi-tô on the 18th and 24th days of adenine feeding when renal impairment was severe. In an examination of the effect of intraperitoneal administration of Onpi-tô extract on RPF, a significant increase was observed on days 6 and 12 (Table II). On day 6, the RPF value was increased from 13.00 ml/min/kg to 17.88 ml/min/kg at the 5-mg level (a 38% change, $p<0.05$) and from 13.00 ml/min/kg to 20.21 ml/min/kg at the 10-mg level (a 55% change, $p<0.05$). A significant increase was also observed on the 12th day. The intraperitoneal administration of 5 mg of Onpi-tô extract caused a 118% increase in RPF as compared with the control rats. Further increase in the dose to the 10-mg level produced a further increase of 172%

Table II Effect of Onpi-tô extract on renal plasma flow.

Day	Material	Dose (mg/100 g B.W.)	RPF (ml/min/kg)
6	Control	-	13.00±2.12
	Onpi-tô	5	17.88±1.14*
	Onpi-tô	10	20.21±2.84*
12	Control	-	6.67±1.34
	Onpi-tô	5	14.55±2.28**
	Onpi-tô	10	18.15±3.52**
18	Control	-	3.94±0.40
	Onpi-tô	5	3.92±0.80
	Onpi-tô	10	4.10±0.53
24	Control	-	1.75±0.43
	Onpi-tô	5	1.88±0.44
	Onpi-tô	10	1.86±0.32

Significantly different from the control value, *$p<0.05$, **$p<0.01$.

in the RPF value. In contrast, RPF in the Onpi-tô extract-treated group on days 18 and 24 showed behavior similar to GFR, no changes being produced. Changes in RBF are shown in Table III. The administration of Onpi-tô extract at a dose level of 5 mg caused an increase in RBF (this variation was not statistically significant), whereas administration of 10 mg of Onpi-tô

Table III Effect of Onpi-tô extract on renal blood flow.

Day	Material	Dose (mg/100 g B.W.)	RBF (ml/min/kg)
6	Control	-	29.84±5.43
	Onpi-tô	5	37.04±3.02
	Onpi-tô	10	44.38±5.21*
12	Control	-	16.44±4.14
	Onpi-tô	5	30.74±7.92
	Onpi-tô	10	37.91±7.85*
18	Control	-	7.55±1.47
	Onpi-tô	5	7.44±1.32
	Onpi-tô	10	8.16±0.49
24	Control	-	2.82±0.79
	Onpi-tô	5	3.02±0.79
	Onpi-tô	10	3.17±0.52

Significantly different from the control value, *$p<0.05$.

extract significantly increased RBF by 49% of the control value ; the RBF value showed a direct correlation with the amount of extract administered. On day 12, administration of 5 mg of extract increased RBF from 16.44 ml/min/kg to 30.74 ml/min/kg, a 87% change. The RBF value produced with an Onpi-tô extract dosage level of 10 mg was significantly higher than that of the control group. Onpi-tô extract at a dosage level of 10 mg produced a significant rise in RBF from 16.44 ml/min/kg to 37.91 ml/min/kg (a 131% change, $p<0.05$). However, on days 18 and 24, there were no significant differences in RBF between the control and Onpi-tô extract-treated groups, at either the 5-mg or 10-mg dosage level.

Discussion

Onpi-tô, which is described in "Bei-Ji-Qian-Jin-Yao-Fang(備急千金要方)," is a prescription composed of Rhei Rhizoma as the main ingredient, together with Aconiti Tuber, Ginseng Radix, Glycyrrhizae Radix and Zingiberis Rhizoma.[13] Of these components, Rhei Rhizoma is classified as a "cold" drug in Chinese medicine, whereas Aconiti Tuber, Ginseng Radix and Zingiberis Rhizoma are classified as "warm" drugs. The latter materials are considered to neutralize the cold property of Rhei Rhizoma. With regard to the pharmacology of Rhei Rhizoma, laxative, antibiotic, astringent, stomachic and cholagogue actions have generally been noted.[14] In addition to

these properties, we have found that this crude drug has a nitrogen metabolism-improving action.[15] Aconiti Tuber has long been known for its cardiotonic action, facilitating systemic circulatory efficiency and improving cardiovascular function.[16] Ginseng Radix activates various metabolic systems, improves anemia, exerts a cardiotonic action and dilates peripheral blood vessels.[17] Zingiberis Rhizoma facilitates blood circulation,[18] and Glycyrrhizae Radix has antidotal, corticoid-like, anti-inflammatory and antiallergic actions.[19] In the previous paper, we reported that Rhei Rhizoma markedly decreased or eliminated serum urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid in rats given adenine, thus improving metabolism under conditions of renal failure.[20-22] On the other hand, we demonstrated that Ginseng Radix also decreased the blood methylguanidine level and increased blood flow in the renal tissue.[23]

In the present study, when Onpi-tô was administered to rats with relatively mild renal failure induced by 6 or 12 days of adenine ingestion, both RPF and RBF increased significantly, and GFR tended to increase, showing activated renal function. However, no such actions were observed in rats with moderate or severe renal failure caused by 18 or 24 days of adenine ingestion, and thus the actions of Onpi-tô were attenuated with the rapid progression of renal failure. This seems to indicate that Onpi-tô acted on the remaining nephrons, and that its actions diminished along with a decrease in the number of functioning nephrons, finally resulting in disappearance of such actions due to termination of renal function. Although Onpi-tô is a prescription containing 43% Rhei Rhizoma as the main ingredient, Rhei Rhizoma alone does not exert any of the renal function-improving actions of Onpi-tô (unpublished data). Previous experiments have revealed that Rhei Rhizoma is involved in the improvement of nitrogen metabolism in the living body under conditions of renal failure,[20-22] and that the activation of renal function by Onpi-tô is probably based on the action of warm drugs such as Aconiti Tuber, Ginseng Radix and Zingiberis Rhizoma, rather than Rhei Rhizoma. These warm drugs were considered to neutralize the "cold" property of Rhei Rhizoma by exerting a general body-warming action (facilitating blood circulation), maintaining the homeostasis of the kidney. Supporting this speculation, Onpi-tô was found to have effects on renovascular parameters such as RPF and RBF.

On the other hand, we have found that Salviae Miltiorrhizae Radix, a representative herb medicine for improving blood flow and preventing blood stasis, facilitates renal function in a similar way to Onpi-tô when intraperitoneally administered.[24,25] In particular, this drug increased GFR even in rats with considerably advanced renal failure after 18 days of adenine administration. The levels of urinary excretion of urea and creatinine were also significantly increased, suggesting facilitated glomerular function. From these findings, it was considered that Salviae Miltiorrhizae Radix acts on both filtration function and the renovascular system, whereas Onpi-tô mainly acts on the renovascular system. However, the nitrogen metabolism-improving effect of orally administered Onpi-tô in the living body was greater than that of Salviae Miltiorrhizae Radix. In particular, the effects of Onpi-tô on methylguanidine and guanidinosuccinic acid, *i.e.*, uremic toxins, were conspicuous. From the clinical viewpoint, our study group has clarified that Onpi-tô often improves the subjective symptoms of patients with chronic renal failure, such as general malaise, cold sensation in the extremities and general coldness, which are considered to be "the cold syndrome" in Chinese medicine.[26] Therefore, it seems that Onpi-tô could be a prescription for a new type of conservative treatment, which delays the progression of renal failure through an action mechanism different from that of Salviae Miltiorrhizae Radix or other widely used therapies such as low-protein and high-calorie therapy, essential amino acid therapy[27] and administration of activated charcoal[28] or lactulose.[29]

和文抄録

あらかじめアデニン食を投与し，腎不全を惹起

させたラットに温脾湯エキスを投与し，腎機能パラメータに及ぼす影響を検討した。腎血漿流量（RPF），腎血流量（RBF）に対し温脾湯（5 mg, 10 mg/100 g体重）は6日，12日目でいずれも有意に増加し，腎機能の亢進作用を認めた。しかしこれら作用は18日，24日目ではいずれも認められなかった。糸球体濾過値（GFR）に対し温脾湯（5 mg, 10 mg 投与群）は6日，12日目で増加傾向，18日，24日目ではRPF，RBFと同様，その作用は認められなかった。

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