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Effects of Ompi-to on the urinary levels of prostaglandin and kallikrein in rats with renal failure

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Abstract

The effects of Ompi-to administration on the urinary excretion of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), and also kallikrein activity, were investigated by administering both adenine and Ompi-to extract to rats. In rats given Ompi-to at a dose of 80 mg/rat/day for 12 or 24 days, the urinary excretion of PGE_2 increased significantly. A significant increase in 6-keto-$PGF_{1\alpha}$ was found in rats given 80 mg of Ompi-to for 12 days. On the other hand, the activity of kallikrein in urine increased markedly and significantly in rats given 40 mg or 80 mg of Ompi-to for 12 or 24 days.

Key words Ompi-to (Onpi-tô), prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, kallikrein, rat.

Abbreviations 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PGE_2, prostaglandin E_2 ; PGI_2, prostaglandin I_2 ; Ompi-to (Wen-Pi-Tang), 温脾湯.

Introduction

Our previous experiments have demonstrated that Ompi-to improves the decreased renal function in rats with renal failure.[1] It is known that various autacoids affect the hemodynamics of the kidney, and that many of endogenous vasoactive substances are produced in the kidney. In the present study, the effects of Ompi-to administration on the levels of prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ and kallikrein, which are known to increase along with vasodilation, were determined. This paper reports the interesting findings that were obtained.

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, which produced experimental renal failure in the animals.[2-6] During the adenine-feeding period, an aqueous solution of Ompi-to was administered orally at doses of 40 and 80 mg/rat/day, respectively, for 24 successive days as drinking water, while control rats received tap-water. On the 11－12th and 23－24th days of the experimental period, individual 24-hr urine samples were collected in separate Erlenmeyer flasks. Throughout the experimental period, there were no statistically significant differences between the control and Ompi-to-treated groups with regard to changes in body weight. The food intake was essentially proportional to weight change. In addition, daily intake of drinking water showed no appreciable changs in each group. No case of diarrheal symptoms was found. The levels of

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serum constituents in normal rats were urea nitrogen 16.3±1.1 mg/dl and creatinine 0.73±0.01 mg/dl. Methylguanidine was not detectable. In contrast, the urea nitrogen and creatinine values on the 24th experimental day in adenine-fed rats had increased to 127.0±11.49 mg/dl and 3.92±0.19 mg/dl, respectively. At the same time, methylguanidine was abnormally high at 16.5 μg/dl. Six rats were used for each experimental group. Values were expressed as means±S.E.

Chemicals : A [^{125}I]prostaglandin E_2 RIA kit was provided by New England Nuclear (Boston, MA, U.S.A.). A [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ RIA kit was purchased from Amersham Co. (Amersham, U.K.). DL-Val-Leu-Arg *p*-nitroanilide was obtained from Sigma Chemical Co., U.S.A.

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.

Prostaglandin assay : Prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in urine were measured by radioimmunoassay as reported elsewhere.[7-9] Prostaglandins in urine samples were extracted with an octadecyl silica mini-column (Analytichem International, Harbor City, U.S.A.). Representative recoveries by this extraction procedure were estimated to be PGE_2, 95% ; 6-keto-$PGF_{1\alpha}$, 93%. The eluate from the octadecyl column was evaporated under a N_2 stream ; the residue was redissolved in EtOAc and separated on a silica gel G plate (Whatman Chemical Separation Inc., Clifton, U.S.A.) using a solvent system of EtOAc : isooctane : AcOH : H_2O=180 : 50 : 20 : 100 (v/v/v/v), upper layer. Prostaglandin standards were run in parallel with the samples and the positions of standards were determined by exposure of the plate to iodine vapor. Silica gel corresponding to areas containing PGE_2 or 6-keto-$PGF_{1\alpha}$ was scraped off, the metabolites were extracted with MeOH-ether (1 : 1, v/v) and then analyzed using an RIA kit. Recoveries of these metabolites by this extraction procedure were 85% and 82%, respectively. The radioactivity was determined using an Aloka liquid scintillation spectrometer, model LSC-900, or an Aloka well gamma system, model ARC-500. The final recovery of the [^{125}I]PGE_2 or [^{3}H]6-keto-$PGF_{1\alpha}$ initially added to urine samples was 81% and 76%, respectively. Appropriate corrections for recovery rates were made when calculating the concentration of prostaglandins.

Kallikrein assay : The activity of kallikrein was assayed according to the method of Amundsen *et al*.[10] The assay mixture contained 100 μl of 1 mM DL-Val-Leu-Arg *p*-nitroanilide and 500 μl of Tris-HCl buffer (pH 8.2). The reaction was started by addition of a suitably diluted urine sample to the assay mixture. After incubation at 37℃ for 30 min, the reaction was terminated by addition of 100 μl of 50% AcOH. The precipitate formed was removed by centrifugation after the mixture had been left to stand. An aliquot was pipetted off and the kallikrein activity in the supernatant was determined spectrophotometrically by measuring the optical density at 405 nm. Kallikrein activity, calculated from the difference in extinction between the sample and blank, was expressed as units/24 hr, 1 unit being the amount of kallikrein that cleaves 1 μM of substrate per minute of incubation time.

Statistics : The significance of differences between the normal and renal failure rats treated or non-treated with Ompi-to was tested using Student's *t* test.

Results

Changes in urinary excretion of prostaglandins in rats given the adenine diet together with oral Ompi-to extract administration are shown in Table I. Among rats given 12 days of the adenine diet, urinary excretion of PGE_2 was 17.47 ng/24 hr in controls, whereas the values were significantly higher by 29% and 57% in rats given

Table I Effect of Ompi-to extract on urinary prostaglandin excretion.

Day	Group	Dose (mg/rat/day)	PGE_2 (ng/24 hr)	6-Keto-$PGF_{1\alpha}$ (ng/24 hr)
12	Normal rat	—	29.72±2.21	23.47±1.81
	Renal failure rat			
	Control	—	17.47±2.75[b]	18.43±2.06[a]
	Ompi-to	40	22.59±3.59	22.52±3.35
	Ompi-to	80	27.37±3.29[#]	30.00±4.80[#]
24	Normal rat	—	28.84±1.76	24.40±3.03
	Renal failure rat			
	Control	—	5.14±1.20[c]	10.30±1.55[c]
	Ompi-to	40	7.24±0.54[c]	12.24±1.39[b]
	Ompi-to	80	9.77±1.32[c],[#]	12.66±1.15[b]

PGE_2, prostaglandin E_2 ; 6-Keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$. Values are means±S.E. of 6 rats. Statistical significance : a) $p<0.05$, b) $p<0.01$, c) $p<0.001$ *vs.* normal rat, $^{\#}p<0.05$ *vs.* renal failure control rat.

40 mg and 80 mg of Ompi-to extract, respectively. Similar changes produced by Ompi-to extract administration were observed on day 24, even though control rats showed a very low PGE_2 excretion corresponding to 18% of that for normal rats. The PGE_2 excretion in rats of the Ompi-to extract-treated group increased from 5.14 ng/24 hr to 7.24 ng/24 hr at the 40-mg level (a 41% change) and from 5.14 ng/24 hr to 9.77 ng/24 hr at the 80-mg level (a 90% change, $p<0.05$). The 6-keto-$PGF_{1\alpha}$ level also decreased gradually with the progress of renal failure, in a similar manner to the case of PGE_2. In rats given 12 and 24 days of the adenine diet, urinary excretion of 6-keto-$PGF_{1\alpha}$ was 18.43 and 10.30 ng/24 hr, respectively. On the other hand, rats given 40 mg of Ompi-to extract for 12 days showed an increase in the urinary excretion of 6-keto-$PGF_{1\alpha}$ (this variation was not statistically significant), whereas administration of 80 mg of Ompi-to extract significantly increased the urinary excretion of 6-keto-$PGF_{1\alpha}$ by 63% of the control value. On day 24, there were no significant differences in urinary 6-keto-$PGF_{1\alpha}$ excretion between the control and Ompi-to extract-treated groups, at either the 40-mg or 80-mg dosage level. The activity

Table II Effect of Ompi-to extract on urinary kallikrein activity.

Day	Group	Dose (mg/rat/day)	Kallikrein activity (mU/24 hr)
12	Normal rat	—	1330±110
	Renal failure rat		
	Control	—	84±20[a]
	Ompi-to	40	203±31[a],[##]
	Ompi-to	80	356±50[a],[###]
24	Normal rat	—	1562±143
	Renal failure rat		
	Control	—	19±5[a]
	Ompi-to	40	153±45[a],[#]
	Ompi-to	80	219±50[a],[##]

Values are means±S.E. of 6 rats. Statistical significance : a) $p<0.001$ *vs.* normal rat, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs.* renal failure control rat.

of kallikrein in urine was maintained within the range of 1330 to 1562 mU/24 hr in normal rats, whereas in the adenine-administered rats, it decreased markedly with the progress of renal failure, becoming approximately 6% of the normal value by the 12th day following the start of administration. The level then fell gradually in the range of 19±5 mU/24 hr on day 24 (Table II). When the effect of oral administration of Ompi-to extract on kallikrein activity was examined, a significant increase was observed on days 12 and 24. On day 12, the kallikrein activity was increased from 84 mU/24 hr to 203 mU/24 hr at the 40-mg level (a 142% change, $p<0.01$) and from 84 mU/24 hr to 356 mU/24 hr at the 80-mg level (a 324% change, $p<0.001$). A significant increase was also observed on the 24th day. Oral administration of 40 mg of Ompi-to extract caused a 705% increase in kallikrein activity as compared with the control rats. Further increase in the dose to 80 mg produced a further increase of 1053% in the urinary kallikrein activity.

Discussion

Among various humoral factors which may play an important role in the regulation of hemodynamics in the kidney, prostaglandin is thought to be an important modulator of glomerular blood flow and filtration rate, since production of this hormone has been observed in cells at various sites of the kidney including interstitial cells, uriniferous tubules, glomeruli and arterioles. PGE_2 and prostaglandin I_2 (PGI_2, an active form of 6-keto-$PGF_{1\alpha}$) are thought to act mainly on mesangial cells in the glomeruli and the small vessel system of the kidney causing vasodilation, and to operate antagonistically against vasoconstriction produced by TXA_2 (an active form of TXB_2), thus playing a role in homeostasis of the hemodynamics of the glomeruli and kidney as a whole.[11] On the other hand, some metabolites of arachidonic acid produced in the kidney are excreted into urine or renal venous blood, being still active or subsequently metabolized further. Many enzymes involved in PGE_2 metabolism are present in the renal cortex. It is considered that PGE_2 excreted into urine mostly originates from the renal medulla.[12] Although 6-keto-$PGF_{1\alpha}$ in urine is generally considered to mainly reflect the production of PGI_2 in the kidney, it is also possible that this substance is derived from circulating blood. Thus, the origin of this substance is not fully clear.[13] However, the use of urine samples in determination of arachidonic acid metabolites produced in the kidney seems to be useful, despite the fact that some minor problems are involved. It has previously been reported that the urinary levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ decrease gradually with the progress of renal failure in rats given adenine.[6] In the present study, there was also a 79% decrease in PGE_2 at 24 days, and 6-keto-$PGF_{1\alpha}$ also showed a significant decrease, although the degree of the decrease was smaller than that of PGE_2. However, the urinary levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in rats given adenine in combination with Ompi-to at a daily dose of 80 mg were significantly increased at 12 days, and there was a similar increasing tendency even in rats given Ompi-to at a daily dose of 40 mg. In rats given 80 mg of Ompi-to, the PGE_2 level at 24 days was also increased significantly, indicating that Ompi-to has some effect on the initiation of the arachidonic acid cascade. It has been shown previously that PGE_2 and 6-keto-$PGF_{1\alpha}$ not only increase renal blood flow by dilating the renal blood vessels, but also cause relaxation of mesangial cells, suppression of immune function and inhibition of platelet aggregation.[14] From the results of the present study, it is presumed that Ompi-to exerts a protective action by interfering with the progression of renal disorder. Kunze *et al*.[15] and Vargaftig *et al*.[16] have demonstrated the following mechanism of prostaglandin release : Kallikrein activates phospholipase A_2, resulting in enhanced production of arachidonic acid and prostaglandin synthesis. On the other hand, in an experiment using perfused rat kidney specimens, Roblero *et al*.[17] have shown that kallikrein in urine is derived from the kidney. It has also been reported that this substance is produced in conjugated uriniferous tubules.[18] Urinary kallikrein excretion is considered to be a valid index of renal kallikrein

synthesis. In the present study, the urinary excretion of kallikrein in rats given Ompi-to increased with an increase in the dose of the drug. Although the degree of the increase in kallikrein was not consistent with than in prostaglandin, the pattern of the increase was similar to those for PGE_2 and 6-keto-$PGF_{1\alpha}$. This clearly indicates that Ompi-to activates the kallikrein-prostaglandin system. We have already reported that Ompi-to improves the decreased renal function under conditions of renal failure.[1] On the basis of the present findings, it seems that Ompi-to induces dilatation of renal blood vessels, increases the renal blood flow and enhances glomerular filtration by improving the renal circulatory state through activation of kallikrein and promotion of the production and secretion of PGE_2 and 6-keto-$PGF_{1\alpha}$. We intend to further study the effects of other autacoids on renal circulation.

和文抄録

温脾湯エキスをアデニン投与と同時に投与し，尿中プロスタグランジン E_2 (PGE_2)，6-ケト-プロスタグランジン $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) 排泄量並びにカリクレイン活性に及ぼす影響を検討した。PGE_2 排泄量は温脾湯 80 mg/rat/day を12日間並びに24日間投与したラットにおいて有意に増加し，6-keto-$PGF_{1\alpha}$ は12日間投与したラットの 80 mg 投与群において有意な増加作用が認められた。一方，尿中カリクレイン活性は12日間，24日間投与したラットの 40 mg, 80 mg 投与群において著しく有意に増加していた。

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