The effect of Onpi-tô on urinary excretion of methylguanidine in rats with chronic renal failure

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Abstract

In rats given an adenine diet, urinary methylguanidine excretion showed a significant increase during the feeding period as compared with rats on a normal diet. However, rats given Onpi-tô at the time of adenine administration showed a decrease of urinary methylguanidine excretion. The total amount of urinary methylguanidine excreted throughout the entire experiment was lower in the Onpi-tô-treated rats than in control rats (458.2 μg/30 days *vs.* 674.2 μg/30 days, degree of reduction, 32%).

Key words Onpi-tô, chronic renal failure, methylguanidine, rat
Abbreviation Onpi-tô (Wen-Pi-Tang), 温脾湯

Introduction

Methylguanidine is known to be present in the serum and urine of patients suffering from chronic renal failure, and produces complications as a potent uremic toxin. Giovannetti *et al.* investigated the toxicity of methylguanidine and found various uremia-like symptoms such as anemia, anorexia, vomiting, diarrhea, hyperexcitability, myoclonus, paresis, convulsion, arrhythmia, gastro-intestinal disturbances, *etc.*[1-3] It has hitherto been reported that administration of essential amino acids under stringent protein restriction as a conservative therapy for renal failure promotes a significant decrease in methylguanidine.[4] On the other hand, experimental results that we have obtained using rats with chronic renal failure induced by an adenine diet have shown that the rhubarb-containing prescription Onpi-tô (Wen-Pi-Tang) remarkably lowers the levels of serum urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid, and other substances associated with chronic toxemia.[5-7] Interesting findings were obtained with regard to guanidino compounds. Rats which were given Onpi-tô appeared to exhibit a gradual decrease of the serum methylguanidine level with increasing dosage. The present paper describes further studies which have been conducted on the effect of Onpi-tô on the urinary excretion of methylguanidine.

Materials and Methods

Animals and treatment : Male rats of the JCL : Wistar strain, initially weighing 110－120 g, were placed in a metabolic chamber under a conventional lighting regimen with a dark-light period. The animals were given a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan) for one week after arrival. They were then fed

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ad libitum on an 18% casein diet containing 0.75% adenine for 30 days. The 18% casein diet had the following composition (in 100 g) : casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[8] 4 g, vitamin mixture[8] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. The procedure of adenine feeding produced experimental chronic renal failure.[9] During the adenine feeding period, Onpi-tô was allowed *ad libitum* at a concentration of 1 mg/ml in drinking water, while control rats received only tap water. The dose of Onpi-tô was about 30 mg/rat/day during the experimental period. Individual 48-h urine samples were each collected in a 100-ml Erlenmeyer flask. There were no statistically significant differences between the control and Onpi-tô-treated groups with regard to body weight. The food intake of the two groups was essentially proportional to weight change throughout the experimental period. The levels of serum constituents in normal rats were as follows : 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 30th experimental day in adenine-fed rats had increased to 150.0±17.7 mg/dl and 2.34±0.10 mg/dl, respectively. At the same time, methylguanidine was abnormally high at 14.5 μg/dl. Continuous and simultaneous administration of adenine and Onpi-tô resulted in a reduction of serum urea nitrogen from 150.0±17.7 to 90.9±7.9 mg/dl ($p < 0.001$). The level of methylguanidine was 5.5 ± 0.4 μg/dl, which was significantly lower than that of the control rats. A slight decrease (8 % compared to the control) was observed in the level of serum creatinine.

Onpi-tô : The Onpi-tô preparation was the same as that previously described.[5] 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 as follows : the above-mentioned crude drugs were boiled gently in 1000 ml of water for 65 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%.

Analysis : For the determination of methylguanidine, urine was deproteinized by addition of TCA (final concentration, 10%). The supernatant obtained by centrifugation at 3000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system. A fluorescence spectrometer, model FP-210 (excitation 365 nm, emission 495 nm ; Japan Spectroscopic Co., Tokyo, Japan) was used for detection of the substances on the column.[10]

Results

Adenine-fed rats exhibited a significant increase in urinary methylguanidine throughout the experimental period. As shown in Fig. 1, the quantity of urinary methylguanidine excreted by the rats fed on an adenine diet rose to 12.3－104.6 μg/2 days compared with 5.2－11.1 μg/2 days for normal rats on days 2－30. Methylguanidine values were 2.3 times greater than those of normal rats on the 10th experimental day, 5.0 times greater on the 20th experimental day, and 9.4 times greater on the 30th experimental day. An abnormally high value of about 100 μg/2 days was noted on days 26－30. In contrast, rats which were given Onpi-tô at the time of adenine administration showed a decrease of urinary methylguanidine excretion, especially on days 18－30, when the level decreased to 34.0－53.2 μg/2 days compared with 48.4－104.6 μg/2 days in control rats. The total urinary methylguanidine level in the Onpi-tô-treated group throughout the experimental period also exhibited an appreciable decrement of about 32% in comparison with that in the control group (458.2 μg/30 days *vs.* 674.2 μg/30 days).

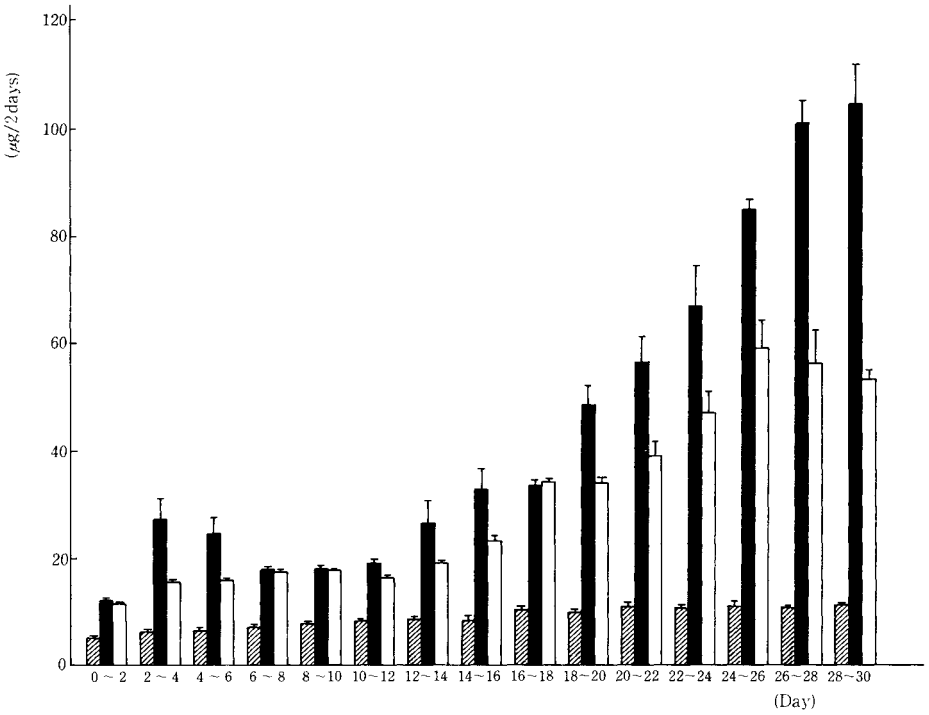

Fig. 1 Urinary excretion of methylguanidine.
▨, normal rat ; ■, chronic renal failure rat (control group) ; □, chronic renal failure rat (Onpi-tô-treated group). Values are means ± S.E. of 6 rats.

Discussion

Since studies on uremic toxins were first undertaken, a raised concentration of serum methylguanidine has been found in patients with uremia.[11] Many toxic effects of methylguanidine have been reported in both dogs and patients with chronic renal failure.[1-3] On the other hand, some studies have demonstrated increased urinary excretion of methylguanidine in rats with chronic renal failure.[12] It is generally accepted that the urinary excretion of methylguanidine is indicative of its metabolic production, since its fecal excretion is negligible.

Among forms of conservative treatment for chronic renal failure, low-protein diets are widely used as a means of dietary therapy for uremia and satisfactory results in the relief of symptoms are generally reported.[13] However, protein malnutrition in uremic patients is thought to bring about other factors which are known to lead to a negative nitrogen balance : inadequate caloric supply, intercurrent infections, proteinuria, dialysis, *etc.*[13] As a result, protein depletion is the limiting factor of dietary therapy. In our present experiment, the reduced urinary methylguanidine level attained by Onpi-tô administration under an 18 % protein regimen may suggest the acceptability of a certain protein load and thus the possibility of improving the whole body condition. In addition, our observations in the preceding study suggested that Onpi-tô administration to rats with chronic renal failure decreased serum methylguanidine in a dose-dependent manner.[5] The decrease of methylguanidine level in serum which was reported in the preceding paper[5] and the decrement of urinary methylguanidine excretion in the present study are considered to indicate the inhibition of methylguanidine production by Onpi-tô. This is believed to be the first report of a crude drug which inhibits the production of methylguanidine.

From the above findings, it appears likely that Onpi-tô is able to delay the progress of renal failure by inhibiting the production of methylguanidine, which is known to be a potent uremic toxins, and improving various states of metabolic abnormality, as reported previously.[5-7,14] A recently published case report has indicated that patients with chronic renal failure respond well to Onpi-tô.[15,16] The findings obtained from our laboratory are thought to provide a partial explanation for the improvement of uremia which is observed clinically following administration of Onpi-tô.

和文抄録

腎不全では methylguanidine (MG) 産生が著しく亢進し，尿毒症の多彩な症状発現に関与しているものと考えられている。本研究において尿中排泄量を測定した結果，腎不全の進行とともに著しく増加したMGが，温脾湯投与により明らかに低下する結果が得られ，先に報告したMGの血中レベルの著しい低下から，温脾湯がMG産生抑制作用を有することが明らかとなった。

References

1) Giovannetti, S., Cioni, L., Balestri, P. L. and Biagini, M.: Evidence that guanidines and some related compounds cause haemolysis in chronic uraemia. *Clin. Sci.* **34**, 141-148, 1968

2) Giovannetti, S., Biagini, M., Balestri, P. L., Navalesi, R., Giagnoni, P., deMatteis, A., Ferro-Milone, P. and Perfetti, C.: Uraemia-like syndrome in dogs chronically intoxicated with methylguanidine and creatinine. *Clin. Sci.* **36**, 445-452, 1969

3) Barsotti, G., Bevilacqua, G., Morelli, E., Cappelli, P., Balestri, P. L. and Giovannetti, S.: Toxicity arising from guanidine compounds: Role of methylguanidine as a uremic toxin. *Kidney Int.* **7**, s-299-s-301, 1975

4) Ando, A., Orita, Y., Nakata, K., Tsubakihara, Y., Takamitsu, Y., Ueda, N., Yanase, M. and Abe, H.: Effect of low protein diet and surplus of essential amino acids on the serum concentration and the urinary excretion of methylguanidine and guanidinosuccinic acid in chronic renal failure. *Nephron* **24**, 161-169, 1979

5) Oura, H., Zheng, P. D. and Yokozawa, T.: Effect of Onpi-tô in rats with chronic renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **1**, 209-217, 1984

6) Oura, H., Chung, H. Y., Zheng, P. D., Yokozawa, T., Wakaki, K. and Koizumi, F.: Effect of Onpi-tô administered orally for a long term on rats with chronic renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 365-372, 1985

7) Zheng, P. D., Yokozawa, T. and Oura, H.: Effect of Onpi-tô in adenine-induced chronic renal failure rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 83-88, 1986

8) Harper, A. E.: Amino acid balance and imbalance. Part I. Dietary level of protein and amino acid imbalance. *J. Nutr.* **68**, 405-424, 1959

9) Yokozawa, T., Zheng, P. D., Oura, H. and Koizumi, F.: Animal model of adenine-induced chronic renal failure in rats. *Nephron* **44**, 230-234, 1986

10) Higashidate, S., Maekubo, T., Saito, M., Senda, M. and Hoshino, T.: Rapid and highly sensitive method for the determination of guanidino compounds in body fluids. *Bunseki Kagaku* **33**, 366-370, 1984

11) Pfiffner, J. J. and Myers, V. C.: On the colorimetric estimation of guanidine bases in blood. *J. Biol. Chem.* **87**, 345-355, 1930

12) Orita, Y., Ando, A., Tsubakihara, Y., Mikami, H., Kikuchi, T., Nakata, K. and Abe, H.: Tissue and blood cell concentration of methylguanidine in rats and patients with chronic renal failure. *Nephron* **27**, 35-39, 1981

13) Giovannetti, S., Balestri, P. L., Biagini, M., Menichini, G. and Rindi, P.: Implications of dietary therapy. *Arch. Intern. Med.* **126**, 900-905, 1970

14) Zheng, P. D., Yokozawa, T., Oura, H. and Nakada, T.: Effect of orally administered Onpi-tô to rats with chronic renal failure on blood flow in renal tissue, blood pressure, and hormone levels in blood. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 37-44, 1986

15) Mitsuma, T., Terasawa, K., Yokozawa, T. and Oura, H.: Rhubarb therapy in patients with chronic renal failure (Part 1). *J. Med. Pharm. Soc. WAKAN-YAKU* **1**, 266-278, 1984

16) Mitsuma, T., Yokozawa, T., Oura, H. and Terasawa, K.: Rhubarb therapy in patients with chronic renal failure (Part 2). *Jap. J. Nephrol.*, in press