Effect of various Oriental medical prescriptions on the urinary methylguanidine/creatinine ratio in rats with renal failure

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Abstract

The action of seven Oriental prescriptions given orally to rats with experimental renal failure was examined using the urinary methylguanidine/creatinine ratio as an index. Rats given Dai-saiko-to, Bofu-tsusho-san or Saiko-ka-ryukotsu-borei-to showed a significant decrease or a tendency to have a decrease in this parameter.

Key words methylguanidine/creatinine ratio, hydroxyl radical scavenger, renal failure, Dai-saiko-to, Bofu-tsusho-san, Saiko-ka-ryukotsu-borei-to, rat.

Abbreviations Bofu-tsusho-san (Fang-Feng-Tong-Sheng-San), 防風通聖散; Choto-san (Diao-Teng-San), 釣藤散; Cr, creatinine; Dai-saiko-to (Da-Chai-Hu-Tang), 大柴胡湯; Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan), 八味地黄丸; MG, methylguanidine; OH, hydroxyl radical; 8-OH-dG, 8-hydroxydeoxyguanosine; Ompi-to (Wen-Pi-Tang), 温脾湯; Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), 黄連解毒湯; Saiko-ka-ryukotsu-borei-to (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang), 柴胡加竜骨牡蠣湯; Shichimotsu-koka-to (Qi-Wu-Jiang-Xia-Tang), 七物降下湯.

Introduction

It has gradually become apparent that active oxygen damages biological membranes, protein and DNA, and that this is involved in aging, carcinogenesis and the onset and pathophysiology of rheumatism.[1,2] On the other hand, the involvement of active oxygen in the onset of renal failure has been suggested by Paller *et al.*,[3] Shah and Walker,[4] Diamond *et al.*[5] and Rehan *et al.*,[6] who reported that renal ischemia and renal failure induced by administration of glycerol, puromycin aminonucleoside or antiglomerular basement membrane antibody are improved by treatment with scavengers of the hydroxyl radical (·OH), superoxide anion and hydrogen peroxide. We have demonstrated in previous *in vitro* and *in vivo* experiments that creatol is an intermediate in the process of methylguanidine (MG) production from creatinine (Cr), and that the ·OH is involved in the production of creatol from Cr.[7-10] In an *in vitro* experimental system using isolated liver cells and phorbol myristate acetate-activated neutrophils in the Fenton reaction and other chemical reactions, Aoyagi *et al.*[11-13] demonstrated the production of MG from Cr by active oxygen and proposed indirect measurement of ·OH in terms of the amount MG/Cr ratio. In addition, we have found that, in rats with adenine-induced chronic renal failure, MG production is markedly suppressed after oral administration of Rhubarb and Ompi-to extract, an Oriental medical prescription, and presumed the presence of a ·OH scavenger action of Rhubarb tannin.[14-17] In the present study, we searched for other Oriental medical prescriptions which have a ·OH scavenger action, using the urinary MG/Gr ratio as an index.

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Materials and Methods

Animals and treatments: Male rats of the LWH : Wistar strain, with a body weight of 200-210 g, were placed in metabolic cages and kept at a temperature of 23±1°C under a 12-h dark-light cycle. They were allowed an adaptation period of several days, during which they were fed 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 confirmed previously by histological and biochemical procedures that renal failure was present after 6 days of adenine ingestion.[18-25] Various extracts of Oriental medical prescriptions were dissolved in water, and given to rats orally every day as drinking water with the adenine diet. The dose of each extract was adjusted to 40 mg/rat/day by regulating its concentration in relation to water consumption. Control rats were given a corresponding amount of water. Each rat was placed in a metabolic cage separately, and a 24-h urine sample was obtained every day from day 12 to day 24. Throughout the experiment there were no significant differences in body weight changes between the control and each of the extract-treated rats. The food intake of each rat was essentially proportional to weight change. Six rats were used for each experimental group. Values are expressed as mean±S.E.

Prescriptions: The following prescriptions were obtained from Tsumura & Co., Tokyo, Japan (figures indicate proportions of each ingredient, expressed in parts per whole) : Dai-saiko-to (Bupleuri Radix 6, Pinelliae Tuber 4, Scutellariae Radix 3, Paeoniae Radix 3, Zizyphi Fructus 3, Aurantii Fructus Immaturus 2, Zingiberis Rhizoma 1, Rhei Rhizoma 1), Bofu-tsusho-san (Scutellariae Radix 2, Glycyrrhizae Radix 2, Platycodi Radix 2, Gypsum Fibrosum 2, Atractylodis Rhizoma 2, Rhei Rhizoma 1.5, Schizonepetae Herba 1.2, Gardeniae Fructus 1.2, Paeoniae Radix 1.2, Cnidii Rhizoma 1.2, Angelicae Radix 1.2, Menthae Herba 1.2, Ledebouriellae Radix 1.2, Ephedrae Herba 1,2, Forsythiae Fructus 1.2, Zingiberis Rhizoma 0.3, Talc 3.0, Glauber's salt 1.5), Saiko-ka-ryukotsu-borei-to (Bupleuri Radix 5, Pinelliae Tuber 4, Cinnamomi Cortex 3, Hoelem 3, Scutellariae Radix 2.5, Zizyphi Fructus 2.5, Ginseng Radix 2.5, Ostreae Testa 2.5, Fossilia Ossis Mastodi 2.5, Zingiberis Rhizoma 1), Shichimotsu-koka-to (Paeoniae Radix 4, Angelicae Radix 4, Astragali Radix 3, Rehmanniae Radix 3, Cnidii Rhizoma 3, Phellodendri Cortex 2, Uncariae Ramulus et Uncus 3), Choto-san (Gypsum Fibrosum 5, Aurantii Nobilis Pericarpium 3, Ophiopogonis Tuber 3, Pinelliae Tuber 3, Hoelen 3, Ginseng Radix 2, Ledebouriellae Radix 2, Glycyrrhizae Radix 1, Zingiberis Rhizoma 1, Uncariae Ramulus et Uncus 3, Chrysanthemum morifolium Hemsl 2), Hachimi-jiogan (Rehmanniae Radix 6, Corni Fructus 3, Dioscoreae Rhizoma 3, Alismatis Rhizoma 3, Hoelen 3, Moutan Cortex 2.5, Cinnamoni Cortex 1, Aconiti Tuber 0.5) and Oren-gedoku-to (Scutellariae Radix 3, Coptidis Rhizoma 2, Gardeniae Fructus 2, Phellodendri Cortex 1.5).

Analyses: For the determination of MG and Cr, urine or serum was deproteinized by addition of trichloroacetic acid (final concentration, 10 %). The supernatant obtained by centrifugation at 3,000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatography 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.

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

Results

Table I compares the urinary MG/Cr ratio of the Oriental medical prescription-treated and control rats. The urinary MG/Cr ratio decreased significantly in rats given Dai-saiko-to from day 12 in comparison with control rats. This phenomenon continued until day 24, when the

ratio was 21-40 % lower than that in controls. The MG/Cr ratio tended to decrease from day 12 to day 15 in rats given Bofu-tsusho-san. Thereafter, there was a significant decrease in the ratio from day 16 to day 21, followed by a non-significant tendency to show a decrease on and after day 22. As shown in Table I, rats given Saiko-ka-ryukotsu-borei-to showed a significant decrease in the ratio from day 12 to day 19, the value being 26-43 % lower than that in controls. Although the ratio was lower than in the control group by 20-25 % on and after day 20, this was not significant. In rats given Shichimotsu-koka-to, the MG/Cr ratio was significantly lower, by 30-36 %, than in control from day 12 to day 14. However, the effect was attenuated from day 15, resulting in hardly and decrease in the MG/Cr ratio on and after day 20, as shown in Table I. Rats given Choto-san also showed a decrease in the urinary MG/Cr ratio until day 17, but the effect was as weak as in rats given Shichimotsu-koka-to, the value being similar to that in controls after day 18 or even higher than the control level on days 20-22, On the other hand, the MG/Cr ratio in rats given Hachimi-jio-gan tended to be decreased from day 14 to day 17, but thereafter remained similar to the control level until day 24. Rats given Oren-gedoku-to also showed no marked

Table I Effect of various Oriental medical prescriptions on the urinary MG/Cr ratio.

Day	Control	Dai-saiko-to	Bofu-tsusho-san	Saiko-ka-ryuko-tsu-borei-to	Shichimotsu-koka-to
11-12	2.51±0.29	1.61±0.28[a]	2.02±0.28	1.58±0.12[b]	1.51±0.18[b]
12-13	2.49±0.28	1.67±0.29[a]	1.82±0.26	1.42±0.11[b]	1.68±0.27[a]
13-14	2.69±0.20	2.07±0.22[a]	2.04±0.28	1.77±0.23[b]	1.72±0.21[b]
14-15	3.21±0.27	2.36±0.28[a]	2.65±0.39	2.38±0.29[a]	2.71±0.37
15-16	3.98±0.36	2.60±0.24[b]	2.78±0.31[a]	2.75±0.35[a]	3.21±0.34
16-17	4.36±0.28	2.78±0.34[b]	2.64±0.38[b]	3.01±0.50[a]	3.45±0.45
17-18	5.48±0.35	4.13±0.53[a]	3.67±0.67[a]	3.73±0.66[a]	4.69±0.56
18-19	5.82±0.45	4.19±0.48[a]	3.67±0.56[b]	3.87±0.52[a]	4.73±0.67
19-20	6.13±0.47	4.73±0.50[a]	4.32±0.46[a]	4.57±0.62	5.59±0.62
20-21	6.49±0.29	5.15±0.57[a]	4.92±0.54[a]	5.17±0.77	5.84±0.65
21-22	7.77±0.59	6.00±0.64[a]	6.17±0.65	6.02±0.74	7.23±0.89
22-23	10.11±0.68	7.63±0.72[a]	8.46±0.58	8.06±0.88	9.70±0.62
23-24	11.34±0.82	6.80±0.80[b]	9.32±0.73	9.00±0.87	10.97±0.71

Table I (Continued).

Day	Control	Choto-san	Hachimi-jio-gan	Oren-gedoku-to
11-12	2.51±0.29	1.93±0.22	2.55±0.28	2.64±0.21
12-13	2.49±0.28	1.72±0.27	2.43±0.25	2.72±0.24
13-14	2.69±0.20	1.96±0.26[a]	2.36±0.42	3.27±0.34
14-15	3.21±0.27	2.75±0.22	2.96±0.35	3.68±0.44
15-16	3.98±0.36	3.15±0.44	3.26±0.69	3.98±0.24
16-17	4.36±0.28	3.64±0.42	3.64±0.78	4.00±0.44
17-18	5.48±0.35	5.24±0.38	5.38±0.52	5.46±0.34
18-19	5.82±0.45	5.81±0.42	5.64±0.57	6.51±0.46
19-20	6.13±0.47	7.01±0.62	6.47±0.58	6.43±0.46
20-21	6.49±0.29	7.98±0.72	6.53±0.44	7.27±0.73
21-22	7.77±0.59	8.22±0.54	8.22±0.82	8.06±0.74
22-23	10.11±0.68	9.29±0.66	10.60±0.89	8.91±0.82
23-24	11.34±0.82	11.39±0.61	10.91±0.84	9.77±0.98

Values are expressed as MG/Cr ($\times 10^{-3}$). [a] $p<0.05$; [b] $p<0.01$, *i. e.* significantly different from control value.

Table II Effect of various Oriental medical prescriptions on the urinary Cr excretion.

Day	Control	Dai-saiko-to	Bofu-tsusho-san	Saiko-ka-ryuko-tsu-borei-to	Shichimotsu-koka-to
11-12	5.20±0.49	5.72±0.42	5.40±0.25	5.62±0.55	5.51±0.27
12-13	5.38±0.63	5.76±0.41	5.54±0.20	5.81±0.49	5.64±0.32
13-14	5.39±0.45	5.88±0.46	5.55±0.41	5.77±0.44	5.65±0.45
14-15	5.22±0.56	5.74±0.45	5.38±0.36	5.64±0.49	5.36±0.27
15-16	5.02±0.30	5.52±0.40	5.37±0.42	5.42±0.41	5.17±0.40
16-17	4.98±0.51	5.43±0.41	5.28±0.37	5.30±0.26	5.13±0.38
17-18	4.84±0.38	5.27±0.45	5.08±0.36	5.17±0.31	4.94±0.35
18-19	4.95±0.59	5.24±0.41	5.14±0.30	5.19±0.47	5.06±0.35
19-20	5.04±0.40	5.37±0.37	5.24±0.28	5.21±0.27	5.04±0.22
20-21	4.99±0.53	5.30±0.34	5.18±0.21	5.18±0.32	5.01±0.25
21-22	4.82±0.56	5.11±0.45	4.96±0.33	4.99±0.25	4.80±0.34
22-23	4.83±0.38	5.11±0.46	4.97±0.32	5.01±0.41	4.78±0.33
23-24	4.89±0.48	5.13±0.37	4.98±0.24	5.03±0.31	4.91±0.31

Table II (Continued).

Day	Control	Choto-san	Hachimi-jio-gan	Oren-gedoku-to
11-12	5.20±0.49	5.41±0.48	5.20±0.49	5.13±0.15
12-13	5.38±0.63	5.59±0.24	5.39±0.50	5.03±0.26
13-14	5.39±0.45	5.62±0.34	5.41±0.23	5.15±0.52
14-15	5.22±0.56	5.34±0.16	5.31±0.32	5.01±0.14
15-16	5.02±0.30	5.15±0.23	5.21±0.47	5.11±0.31
16-17	4.98±0.51	5.07±0.24	5.28±0.45	5.08±0.29
17-18	4.84±0.38	4.90±0.34	4.90±0.47	4.92±0.46
18-19	4.95±0.59	4.97±0.35	4.88±0.29	4.75±0.47
19-20	5.04±0.40	4.80±0.27	4.94±0.28	4.83±0.32
20-21	4.99±0.53	4.79±0.24	4.91±0.44	4.79±0.43
21-22	4.82±0.56	4.81±0.21	4.80±0.37	4.82±0.45
22-23	4.83±0.38	4.91±0.37	4.70±0.39	4.74±0.47
23-24	4.89±0.48	4.87±0.28	4.72±0.49	4.92±0.37

Values are expressed as mg/day.

Table III Effect of various Oriental medical prescriptions on the serum MG/Cr ratio and Cr level.

Group	MG/Cr ($\times 10^{-3}$)	Cr (mg/dl)
Control	3.55±0.22	3.43±0.18
Dai-saiko-to	3.11±0.23	3.49±0.15
Bofu-tsusho-san	3.18±0.19	3.49±0.15
Saiko-ka-ryukotsu-borei-to	3.21±0.27	3.47±0.29
Shichimotsu-koka-to	3.51±0.27	3.48±0.28
Choto-san	3.57±0.19	3.42±0.19
Hachimi-jio-gan	3.57±0.20	3.41±0.29
Oren-gedoku-to	3.53±0.24	3.43±0.29

changes in urinary MG/Cr throughout the experimental period (Table I). Changes in urinary excretion of Cr in rats given the various Oriental medical prescriptions are shown in Table II. The urinary Cr excretion showed a slight increase in rats given Dai-saiko-to from day 12 in comparison with control rats. This phenomenon continued until day 24, when the level was 5-10 % higher than that in controls. Rats given Bofu-tsusho-san or Saiko-ka-ryukotsu-borei-to tended to increase in the urinary Cr excretion, as shown in Table II. On the other hand, the urinary Cr excretion in rats given Shichimotsu-koka-to or Choto-san showed no appreciable changes throughout the experimental period. In rats given Hachimi-jio-gan or Oren-gedoku-to, the

urinary Cr excretion tended to decrease as the period of adenine administration lengthened. The serum MG/Cr ratio and Cr level in rats given the extract for 24 days remained nearly unchanged, as shown in Table III.

Discussion

It is known that the production of MG, a potent uremic toxin, is increased markedly along with the progression of renal failure. Since the MG level increases in both and urine, it is speculated that the increase in the blood concentration of MG is attributable to increased production of this substance in the body, rather than to decreased excretion of MG from the kidney. Therefore, MG is considered to be an index useful for the analysis of metabolic disorders and the pathological condition in uremia.[26] On the other hand, the Cr pool in the body is decreased under conditions of renal failure, leading to a decrease in the production of Cr from creatine. However, its excretion is disturbed because of renal hypofunction, leading to an increase in the body Cr concentration.[27] We have reported previously that the precursor of MG *in vivo* is Cr, and demonstrated that ·OH is involved in the production of creatol, an intermediate between Cr and MG.[28, 8, 10] Recently, we determined the level of 8-hydroxydeoxyguanosine (8-OH-dG) in renal tissue, on the basis of the oxidation of a DNA component, deoxyguanosine, to 8-OH-dG by active oxygen, and found a significant, marked increase in this substance in rats with renal failure.[29] Aoyagi *et al.*,[13] Takemura *et al.*[30] and Koda *et al.*[31] have already pointed out the usefulness of expressing the free radical activity, *i.e.*, capability of MG production from Cr, by the urinary MG/Cr ratio. In this connection, in the present study, we investigated the free radical activity of various Oriental medical prescriptions, using the MG/Cr ratio as an index.

Our previous study had demonstrated that Rhubarb and a Rhubarb-containing prescription, Ompi-to extract, suppress the production of MG.[14-17] Among the seven prescriptions used in the present study, Dai-saiko-to and Bofu-tsusho-san contain Rhubarb as a component herb. Rats given Dai-saiko-to showed a significant decrease in the MG/Cr ratio throughout the observation period, and those given Bofu-tsusho-san also showed a significant decrease or a decreasing tendency. However, these effects were much less conspicuous than those of Rhubarb itself or Ompi-to, which contains about 43 % Rhubarb. Dai-saiko-to and Bofu-tsusho-san contain about 4.3 % and 5.5 % Rhubarb, respectively, corresponding to 1/8-1/10 the amount contained in Ompi-to. Since the ingredients other than Rhubarb in Dai-saiko-to are different from those in Bofu-tsusho-san, further investigations of the actions of the respective component drugs and their complex action is necessary. However, the fact that the two Rhubarb-containing prescriptions induced a marked decrease in MG/Cr in comparison with the other five prescriptions suggests the role of Rhubarb as a ·OH scavenger, as in preceding studies. It is worth noting that improvement of chronic nephritis and nephrotic syndrome after administration of Sairei-to or Sho-saiko-to, which contain Bupleurum Root, was reported by Hattori *et al.*,[32] *Yorioka et al.*[33] and Suzuki and Hattori.[34] The involvement of saikosaponin in the suppression of *in vitro* MG production was also demonstrated by Aoyagi *et al.*[11] In our present study, rast given Dai-saiko-to, which contains the highest percentage of Bupleurum Root (26.1 %) among the seven prescriptions examined, showed a significant decrease in urinary MG/Cr on any day between days 12 and 24. In rats given Saiko-ka-ryukotsu-borei-to, which contains 17.5 % Bupleurum Root, there was also a significant decrease in this parameter from day 12 to day 19 and a decreasing trend thereafter until day 24. Thus, Rhubarb- or Bupleurum Root-containing prescriptions, *i.e.*, Dai-saiko-to, Bofu-tsusho-san and Saiko-ka-ryukotsu-borei-to, induced a decrease in urinary MG/Cr, whereas Shichimotsu-koka-to, Choto-san, Hachimi-jio-gan and Orengedoku-to, all of which contain no Rhubarb or Bupleurum Root, exerted only a slight effect on the urinary MG/Cr. These findings suggest that Rhubarb and Bupleurum Root work as ·OH scavengers.

Takahashi *et al.*[35] have observed previously in an *in vitro* experimental system that the same seven prescriptions as those used in the present study worked as ·OH scavengers, the effect being strongest for Hachimi-jio-gan, followed by Oren-gedoku-to and Choto-san in that order. Their results prompted us to design the present study. Since Oriental medical prescriptions are usually taken orally, we adopted the oral route of administration and examined the effects of the prescriptions on the urinary MG/Cr in rats with renal failure, since active oxygen is involved in the onset of this condition. No significant action of the above three prescriptions was found, thus demonstrating an evident discrepancy between *in vitro* and *in vivo* experimental systems. This illustrates the difficulty involved in extrapolating the results of *in vitro* experiments to those in the living body.

和文抄録

7種類の方剤の経口投与による作用を実験的腎不全ラットを用い，尿中 MG/Cr 比を指標に検討した。その結果，大柴胡湯，防風通聖散，柴胡加竜骨牡蠣湯投与群の尿中 MG/Cr 比が有意な低下あるいは低下傾向を示した。

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