# Effects of green tea tannin in dialysis patients

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## Abstract

Green tea tannin (daily dose, 400 mg) administered for 6 months to 50 dialysis patients decreased in the blood levels of creatinine (Cr) and methylguanidine (MG) and also the MG/Cr ratio. A decrease in $\beta_2$-microglobulin and improvement of arthralgia were also noted in some patients. These findings suggest that green tea tannin ameliorates the state of enhanced oxidation in dialysis patients.

**Key words** dialysis, green tea tannin, creatinine, methylguanidine, $\beta_2$-microglobulin, arthralgia, patient.

## Introduction

In Japan, the number of patients on maintenance dialysis has been increasing year by year. Although a probable factor responsible for this increase is the improved vital prognosis of patients on maintenance dialysis owing to advances in this form of treatment, it should also be recognized that the number of new patients at the terminal stage of renal failure in whom dialysis has to be introduced keeps rising.

Although many different diseases can induce chronic renal disease possibly resulting in terminal renal failure, there is no established radical treatment for this condition. Therefore, once a patient has developed chronic renal failure, control and prevention of disease progression and aggravation is critically important.

Focusing our attention on the usefulness of Oriental medicines for drug treatment of chronic renal failure, we have studied their effects, action mechanisms and active constituents.[1-7] After the discovery of creatol, a creatinine (Cr) oxide, we attempted to elucidate the mechanism of Cr oxidation on the basis of the fact that the body is under oxidative stress in renal failure,[8-15] and demonstrated that galenicals containing tannin have antioxidant activity.[16-18] Since tannin is present in large amounts in tea leaves, we investigated the effects of green tea tannin on the kidney. It was found that green tea tannin exerts a depressor effect in rats with renal hypertension and also has antioxidant activity, and that the kallikrein-prostaglandin system, i.e., a renal function- or blood pressure-regulating factor, is involved in these effects.[19-21] Our results also showed that green tea tannin would act directly on renal cells and inhibit the proliferation of mesangial cells.[22,23]

On the basis of the findings obtained from these fundamental studies, we administered green tea tannin to dialysis patients who were in an enhanced oxidative condition, and evaluated its usefulness in their treatment.

## Materials and Methods

*Green tea tannin* : The tea tannin used in this study was Sunphenon (Taiyo Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea, as reported previously.[24] It was composed mainly of (−)-epigallocatechin 3-*O*-gallate (18.0 %), (−)-gallocatechin 3-*O*-gallate (11.6 %), (−)-epicatechin 3-*O*-gallate (4.6 %), (−)-epigallocate-

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chin (15.0 %), (+)-gallocatechin (14.8 %), (−)-epicatechin (7.0 %) and (+)-catechin (3.5 %).

*Experimental protocol* : The subjects were 50 patients receiving chronic hemodialysis at the outpatient clinic of Hasegawa Hospital. They comprised 37 men and 13 women, with a mean age of 50.4 years (range, 27-76 years) and a mean dialysis period of 71.6 months (range, 5-183 months). Chronic glomerulonephritis was the most frequent disease underlying the chronic renal failure, accounting for 31 cases, followed by cystic kidney (6 cases) and malignant hypertension (3 cases). Other underlying diseases include focal glomerular sclerosis, diabetic nephropathy, nephrotic syndrome and gouty kidney. Hemodialysis was given twice or three times a week, and non-high-performance membrane dialyzers were generally used, except when high performance dialysis was required for concomitant carpal canal syndrome or amyloid arthropathy. All the patients were placed on the duration of follow-up for at least 1 month before the study was initiated, and then a dose of 200 mg green tea tannin in a jelly form was given twice daily. Capsules were used instead of jelly in some exceptional cases, considering the patient's taste. Blood samples were collected immediately before dialysis once every month. We obtained consent from all patients before the administration of green tea tannin.

*Analytical methods* : Cr and methylguanidine (MG) were determined using a Japan Spectroscopic liquid chromatograph with a step-gradient system according to the method of Higashidate *et al.*[25] $\beta_2$-Microglobulin ($\beta_2$-MG) was determined by double antibody radioimmunoassay (Phadebas $\beta_2$-micro test, Pharmacia, Uppsala, Sweden). Blood urea nitrogen (BUN), glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, $\gamma$-glutamyl transpeptidase, alkaline phosphatase, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and triglyceride were all measured using an autoanalyzer.

*Statistical analysis* : Values are given as mean± S.E. Statistical differences were considered significant at $p<0.05$, using the paired $t$-test.

## Results

*Cr, MG, MG/Cr ratio*

Table I shows the Cr and MG levels and the MG/Cr ratio obtained during the period of green tea tannin administration. While the Cr level was 13.51 mg/dl at the baseline, it was significantly lower after 3 months of administration, showing almost an 8 % decrease at 5-6 months. A decrease in MG preceded the decrease in Cr, reaching a significant level at 1 month. The MG level was 45.06 $\mu$g/dl at 5 months, which was 20 % lower than the baseline level, 56.43 $\mu$g/dl, thus demonstrating a more potent effect of green tea tannin on this parameter than on Cr. On the other hand, the MG/Cr ratio, $4.12\times10^{-3}$ at the baseline, decreased significantly to $3.86\times10^{-3}$ at 2 months. This suppressive effect continued thereafter throughout the administration period.

When the patients were divided into three groups in terms of the MG level before green tea tannin administration, the group with MG levels of more than 65 $\mu$g/dl (high MG group) showed a significant decrease of MG at 1 month and thereafter, reflecting the results of analysis of the patients as a whole (Fig. 1). The rate of decrease, however, was higher in this group than in patients as a whole at 1 month (11 % *vs.* 5 %), and also remained at high levels ranging from 11 % to 33 % during the administration period. The group of patients who had MG levels of 50-65 $\mu$g/dl at the baseline (middle MG group) began to show a significant decrease at 2 months, while the other

Table I Effect of green tea tannin on serum creatinine, methylguanidine and the methylguanidine/creatinine ratio.

| Duration of treatment (month) | Cr (mg/dl) | MG ($\mu$g/dl) | MG/Cr ($\times10^{-3}$) |
|---|---|---|---|
| 0 | 13.51±0.30 | 56.43±2.67 | 4.12±0.17 |
| 1 | 13.33±0.27 | 53.65±2.30[a] | 3.99±0.14 |
| 2 | 13.28±0.22 | 51.92±2.34[b] | 3.86±0.14[a] |
| 3 | 12.81±0.24[c] | 48.66±1.83[c] | 3.78±0.12[b] |
| 4 | 12.65±0.21[c] | 49.12±1.76[c] | 3.87±0.12[a] |
| 5 | 12.37±0.24[c] | 45.06±1.80[c] | 3.62±0.12[b] |
| 6 | 12.43±0.25[c] | 48.41±2.12[c] | 3.85±0.13[a] |

Significantly different from the pre-treatment value : [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$.

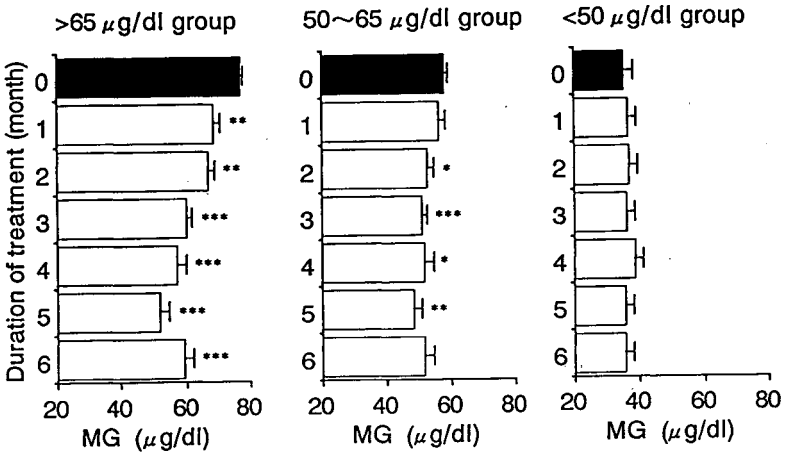


Fig. 1 Effect of green tea tannin on serum methylguanidine. Significantly different from the pre-treatment value : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

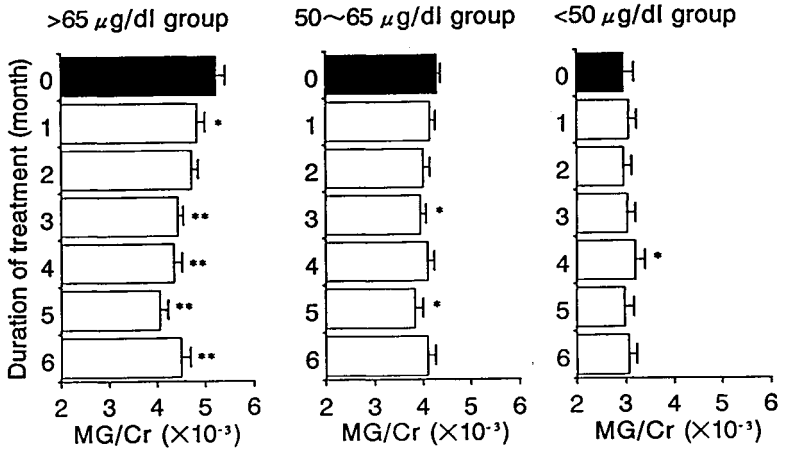


Fig. 2 Effect of green tea tannin on the serum methylguanidine/creatinine ratio. Significantly different from the pre-treatment value : $^{*}p<0.05$, $^{**}p<0.001$.

group, having MG levels of 50 μg/dl below at the baseline (low MG group), showed no significant changes throughout the administration period. The MG/Cr ratio was also decreased markedly in the high MG group (Fig. 2). The typical findings seen in the changes of Cr and MG levels and the MG/Cr ratio are illustrated in Fig. 3.

*$\beta_2$-MG*

Table II shows changes in $\beta_2$-MG during green tea tannin administration. There was a significant decrease in this parameter at every point of measurement except for 2 months after the beginning of administration. The $\beta_2$-MG level was stable at 35-36

Table II Effect of green tea tannin on serum $\beta_2$-microglobulin.

| Duration of treatment (month) | $\beta_2$-MG (mg/dl) |
|---|---|
| 0 | 39.00±1.27 |
| 1 | 34.95±1.08[b] |
| 2 | 37.46±1.30 |
| 3 | 36.36±1.13[a] |
| 4 | 36.11±1.03[b] |
| 5 | 35.65±1.20[a] |
| 6 | 35.38±1.11[a] |

Significantly different from the pre-treatment value : [a]$p<0.01$, [b]$p<0.001$.

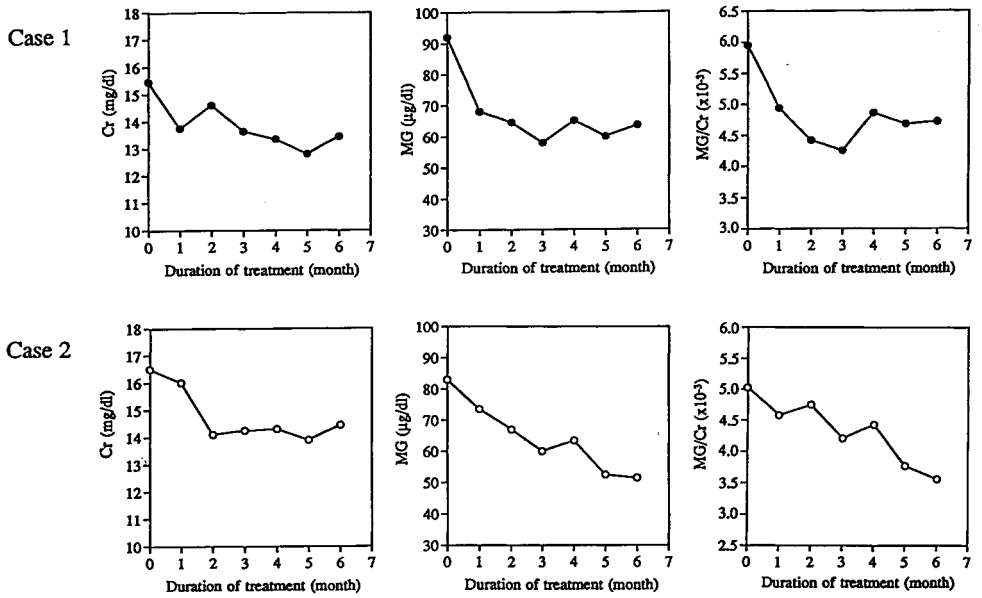


Fig. 3 Time course of the effect of green tea tannin on serum creatinine, methylguanidine and the methylguanidine/creatinine ratio for the two cases.

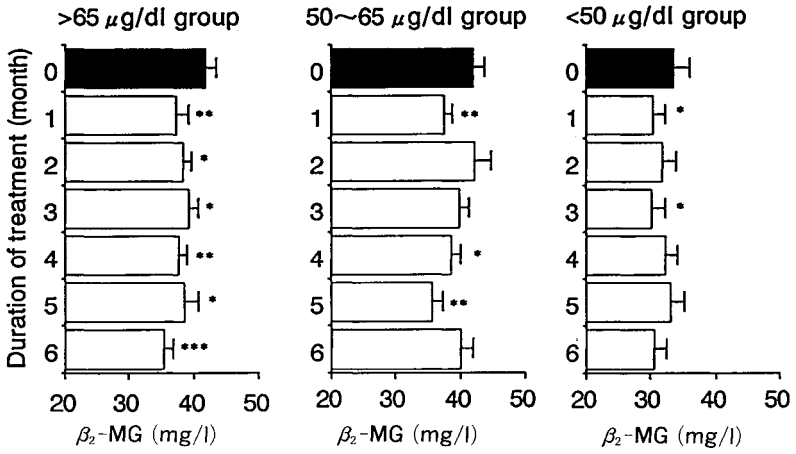


Fig. 4 Effect of green tea tannin on serum $\beta_2$-microglobulin. Significantly different from the pre-treatment value : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

mg/dl after 5 or 6 months of administration. When the effect on $\beta_2$-MG in patients who were on high-performance dialysis was compared with that in those on non-high-performance dialysis, no particular difference was found. On the other hand, among the three groups divided according to the MG level at the baseline, the high and middle MG groups (>65 μg/dl and 50-65 μg/dl) showed high $\beta_2$-MG levels, and the higher the $\beta_2$-MG level at the baseline, the greater the suppressive effect of green tea tannin on it (Fig. 3).

*BUN*

As shown in Table III, the BUN level was 67.5 mg/dl at the baseline, and it showed no significant changes throughout the 6 months of administration, except for

Table III Effect of green tea tannin on blood urea nitrogen.

| Duration of treatment (month) | BUN (mg/dl) |
|---|---|
| 0 | 67.5±1.4 |
| 1 | 62.4±1.5[a] |
| 2 | 66.0±1.6 |
| 3 | 65.4±1.4 |
| 4 | 65.6±1.7 |
| 5 | 66.6±1.6 |
| 6 | 68.6±1.5 |

Significantly different from the pre-treatment value : [a]$p<0.001$.

*Subjective symptoms*

Effects on joint symptoms were investigated by means of questionnaires (Table V). The site of arthralgia varied according to the patient, the shoulder being most frequent, followed in order by the knee, fingers and coxa. The percentage of patients in whom pain disappeared or was relieved after green tea tannin administration was 100 % among those with pain in the hip and cubitus, although the total number of such patients was small. The corresponding rate was about 55-60 % for pain in the fingers or coxa and about 40 % for pain in the shoulder or knee.

Table IV Laboratory findings.

| | Before | After administration for 6 months |
|---|---|---|
| Body weight (kg) | 57.9±1.3 | 57.8±1.3 |
| GOT (KU) | 9.8±1.3 | 10.6±1.8 |
| GPT (KU) | 8.2±1.3 | 8.9±2.2 |
| $\gamma$-GTP (IU) | 23.8±3.4 | 28.5±5.3 |
| Alkaline phosphatase (KAU) | 6.7±0.4 | 6.7±0.4 |
| Total cholesterol (mg/dl) | 169.0±5.4 | 172.1±6.4 |
| LDL-cholesterol (mg/dl) | 109.0±4.6 | 113.2±5.5 |
| HDL-cholesterol (mg/dl) | 31.1±1.4 | 32.5±1.3 |
| Triglyceride (mg/dl) | 144.6±12.7 | 132.6±12.4[a] |

GOT = glutamate oxaloacetate transaminase, GPT = glutamate pyruvate transaminase, $\gamma$-GTP=$\gamma$-glutamyl transpeptidase, LDL-cholesterol=low-density lipoprotein-cholesterol, HDL-cholesterol=high-density lipoprotein-cholesterol. Significantly different from the pre-treatment value : [a]$p<0.05$.

Table V Arthralgia.

| | Disappearance | Relief | No change |
|---|---|---|---|
| Shoulder | 5 | 1 | 10 |
| Knee | 4 | 1 | 8 |
| Finger | 3 | 4 | 6 |
| Coxa | 5 | 1 | 4 |
| Hip | 5 | 1 | 0 |
| Cubitus | 1 | 0 | 0 |

a significant decrease one month after the start of administration.

*Blood chemistry*

As shown in Table IV, no significant changes were found in any of the blood chemical parameters examined, excluding a significant decrease in triglyceride.

## Discussion

As the period of dialysis is prolonged, removal of $\beta_2$-MG becomes less efficient, residual renal function deteriorates, and MG production increases.[26-28] Prolonged dialysis may thus lead to various complications, e.g., malignant tumors occurring at a high incidence,[29,30] carpal canal syndrome,[31] amyloid arthropathy[32] and immune deficiency.[33] These conditions have been attributed to enhanced oxidative stress during prolonged dialysis. Recent advances in dialysis technology have enabled efficient removal of $\beta_2$-MG and also inhibition of MG production to some extent. However, even when improved methods of dialysis are used, the patient's condition tends to be aggravated after a certain period, resulting in further worsen-

ing of oxidative stress. This has highlighted the importance of constant inhibition of oxidative stress, drawing attention to the need for antioxidant therapy.

We have previously isolated creatol from the urine of patients with chronic renal failure, and found that the pathway of Cr metabolism to MG *via* creatol is a common pathway.[34] We have further demonstrated using *in vitro* and *in vivo* experimental systems that, under conditions where hydroxyl radicals are generated, Cr is oxidized to creatol, and then to MG *via* the unstable intermediates creatone A and creatone B.[8, 10, 13, 15] Since determination of these components is useful for evaluating the pathological condition of renal failure, we measured the increased blood levels of Cr and MG in chronic renal failure to demonstrate the antioxidant activity of green tea tannin, which was administered to patients receiving dialysis.

Cr is frequently used in a clinical setting as a renal function parameter, and is also a precursor involved in the conversion of creatol to MG. The level of this substance was found to be decreased significantly after 3 months of green tea tannin administration, and this effect was maintained until the end of the 6-month administration period. On the other hand, the level of MG began to decrease significantly after 1 month of green tea tannin administration, preceding the decrease in Cr, and showed a decrease of 8-10 $\mu$g/dl at 5-6 months. This suggested that green tea tannin influenced the radicals involved in the production of MG from Cr, in addition to decreasing the amount of Cr. Therefore, we calculated the MG/Cr ratio, and found a significant decrease after 2 months of green tea tannin administration. Thus, the radical-scavenger activity of green tea tannin was demonstrated in this clinical study, as in previous animal experiments.[20, 21, 35]

The effect of green tea tannin on MG was found to be strong in the group of patients who had high baseline MG levels ($>65\,\mu$g/dl), whereas there was no significant change in the group of patients with low baseline MG levels ($<50\,\mu$g/dl, about $35\,\mu$g/dl on average), although no increase in MG occurred throughout the administration period. A similar effect was found in the MG/Cr ratio, the decrease being prominent under the most enhanced oxidative stress.

Aggressive removal of $\beta_2$-MG is desirable to prevent complications of prolonged dialysis including amyloidosis. In this connection, green tea tannin caused a significant decrease at every point of measurement during the 6-month administration period, except for 2 months after the start of administration. When the suppressive effect was analyzed in three groups of patients divided according to the MG level at the baseline, i.e., the severity of oxidative stress, a significant decrease in $\beta_2$-MG was found in the high-MG group during the period of green tea tannin administration. It was also noteworthy that there was a decrease in $\beta_2$-MG, even though non-high-performance dialysis, for which it is difficult to eliminate $\beta_2$-MG, was used.

In 1977, Craddock *et al.*[36] reported that transient leukopenia occurred within 1 h after the start of dialysis, and explained the mechanism responsible as activation of the alternative pathway of complement by the dialyzer and sequestration of polymorphonuclear cells by pulmonary blood vessels through the chemotactic action of the activated complement. On the other hand, it has been reported by Haeffner-Cavaillon *et al.*[37] that complement promotes the production by monocytes of interleukin 1, which in turn increases acute-phase protein in the liver and acts on amyloid precursor to stimulate its conversion to amyloid. It has also been shown that oxygen radicals generated by polymorphonuclear cells or monocytes cause damage to vascular endothelial cells.[28] We demonstrated that administration of green tea tannin suppressed oxidative stress in dialysis patients and ameliorated pain in the hip, cubitus, coxa and finger in some of them. This suggests that green tea tannin also inhibits the production of $\beta_2$-MG and its deposition in tissue. There were no significant changes in blood pressure and other general laboratory parameters or subjective symptoms during the period of green tea tannin administration.

Aggressive removal of $\beta_2$-MG and suppression of free radical activity are required in order to prevent complications of prolonged dialysis including amyloidosis. In this connection, wide use of green tea tannin seems a promising form of treatment. In fact, green tea tannin administration appears to offer the possibility of decreasing the frequency of dialysis in some patients. Research in this field is currently insuf-

ficient, and further investigation of a large number of patients is therefore necessary.

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## 和文抄録

透析患者50例に緑茶タンニン(1日400 mg)を6ケ月間用いたところ，血中クレアチニン，メチルグアニジンレベルの低下とともに，メチルグアニジン/クレアチニン比の低下を認め，また$\beta_2$-ミクログロブリンの低下と一部の患者で関節痛の改善効果を示した。以上の成績は緑茶タンニンが透析患者の酸化亢進状態を改善する可能性を示唆するものである。

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