FOOD QUALITY and CULTURE
Food Quality Culture 2(2): 89~96 (2008)

The Effects of Green Tea Tannin in Rats with Renal Failure Induced by Arginine Diet

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ABSTRACT In order to determine whether green tea tannin ameliorates abnormal arginine metabolism as the result of excessive arginine, we have assessed the effects of the administration of green tea tannin mixture in rats treated 30 days with 2% arginine. In the arginine-treated group, the level of guanidino compounds such as arginine (Arg), guanidinoacetic acid (GAA), creatinine (Cr), methylguanidine (MG) and guanidinosuccinic acid (GSA), nitric oxide, urea, protein and glucose increased significantly in the serum, urine and kidney, whereas the oxygen species-scavenging enzymes of kidney were reduced as compared with the non-arginine-treated group. By way of contrast, the administration of green tea tannin reduced blood urea nitrogen and serum creatinine, and reduced the urinary excretion of guanidinoacetic acid, creatinine, and $NO_2^-+NO_3^-$. The increased levels of urinary urea, protein and glucose in the arginine-treated group were also lowered by the administration of green tea tannin. In these groups, the activities of superoxide dismutase and catalase in the kidney were increased, thereby suggesting the involvement of radicals in the normalizing of kidney function. These results show that the abnormal renal function induced by the adminstration of excessive arginine in rats may be restored by treatment with green tea tannin.

KEYWORDS: green tea tannin; renal failure; arginine diet; guanidino compounds; nitric oxide

INTRODUCTION

Green tea contains many types of tannin, and these tannins are known to exert a variety of physiological effects. It has been reported that green tea tannins evidence free radical-scavenging activity *in vitro* and *in vivo* (Yokozawa et al 1998), and inhibit peroxynitrite formation (Chung et al 1998). Tannins also exhibit blood methylguanidine-decreasing effects in cases of renal failure (Yokozawa et al 1997), and suppress the proliferation of mesangial cells (Yokozawa et al 1993) and renal hypertension (Yokozawa et al 1994); these effects are most conspicuous with (-)-epigallocatechin 3-*O*-gallate and (-)-epicatechin 3-*O*-gallate.

Arginine performs a function as a substrate for protein synthesis. In addition, arginine is a precursor of nitrite oxide and creatinine, and participates as arginyl-tRNA in the process of ubiquitin-dependent protein degradation (Cynober et al 1995). The physiological requirements for arginine in tissues and organs are generally supplied by endogenous synthesis and by diet. In the immature rat, guinea pig, cat, dog, chicken, rabbit, and pig, endogenous arginine is insufficient for the maintenance of normal growth and function, and thus these animals require dietary arginine (Castillo et al 1993; Visek 1986). In particular, it has been reported that arginine-deficient diets induced hyperammoniema, vomiting, tremors, and hyperglycemia (Visek 1986). It was also reported that arginine prevented hemorrhage-induced vasconstruction (Benyo et al 1998) and hypercholesterolemia (Andrade et al 1998), and evidenced antitumor activity (Takeda et al 1975). However, the amount of dietary arginine necessary for humans remains to be clearly determined. In the case of liver disease and genetic errors of urea-cycle enzyme activity, endogenous arginine synthesis tends not to be sufficient, and thus dietary arginine is required (Visek 1985). On the other hand, excessive administration of L-arginine has been previously reported to induce pancreatitis in rats (Czako et al 1998; Mizunma et al 1984; Varga et al 1997), enhance injuries from hypoxia/reoxygenation, and increase urinary excretion NO (Barri and Wilcox 1998; Suto et al 1995) and MG (Orita et al 1978).

Although arginine is a crucial amino acid in the body,

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Received September 5, 2008; Revised October 6, 2008;
Accepted October 15, 2008

excessive doses can induce pathogenesis. Therefore, in the current study, we have attempted to assess the influence of excessive arginine in the serum, urine and kidneys, and to assess the effects of the administration of green tea tannin.

MATERIALS AND METHODS

Green tea tannin mixture

The green tea tannin mixture, green tea catechin 45, was purchased from Taiyo Kagaku Co. (Yokkaichi, Japan). It was composed of epigallocatechin (93.36 mg), epicatechin (30.75 mg), epigallocatechin 3-*O*-gallate (226.85 mg), epicatechin 3-*O*-gallate (70.93 mg), catechin (421.89 mg) and caffeine (112.98 mg).

Animal and treatment

Male wistar rats (120-130 g) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). They were maintained in a wire-bottomed cage under a conventional lighting regimen with dark night conditions. The room temperature and humidity were automatically controlled to 25°C and 60%. After several days of adaptation, they were divided into four groups, avoiding any intergroup difference in body weight gain. The normal diet was composed as follows (per 100 g): casein 18 g, sucrose 15 g, α-cornstarch 57.9 g, soybean oil 2 g, salt mixture 4 g, vitamin mixture 1 g, cellulose powder 2 g, and choline chloride 0.1 g. The control diet contained 2% arginine in the 18% casein diet, and green tea tannin was administered at a dosage of 50 or 100 mg/kg of body weight/day, respectively. All groups were orally administered diet with or without green tea tannin for 30 days. Urine specimens were collected for 1-2 days before the rats were sacrificed. Blood samples were obtained by cutting the carotid artery, and the kidneys were subsequently extirpated from each rat.

Determination of guanidino compounds

Blood and urine were collected in conical centrifuge tubes, and the supernatants were immediately acquired via centrifugation. Urea nitrogen was determined using a commercial reagent (BUN Kainos, obtained from Kainos Laboratories, Inc., Tokyo, Japan). For the determination of Arg, GAA, Cr, MG, and GSA, the serum, urine, and kidney samples were deproteinized via the addition of trichloroacetic acid (final concentration, 10, 10, and 1%, respectively). The supernatant obtained via 10 min of centrifugation at 3,000 rpm was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system. A fluorescence spectrometer (excitation 365 nm, emission 495 nm, model FP-210, Japan Spectroscopic Co., Tokyo, Japan) was used for the substances on the column (Higashidate et al 1984).

Determination of nitrite and nitrate

Nitrite (NO_2^-) and nitrate (NO_3^-) were measured with a NOX measuring system, ENO-10 (Eicom Co. Ltd., Tokyo, Japan).

Determination of urine constituents

Urine components were determined as follows; urea via Archibald's method (1945); protein via the sulfosalicylic acid method (Sakagisi 1968); and glucose via the method of Momose et al (1963).

Enzyme assay

The kidney was homogenized with a 9-fold volume of ice-cold physiological saline, and the activities of enzymes in the homogenate were determined. The activity of superoxide dismutase (SOD) was measured according to the nitrose acid method developed by Elstner and Heupel (1976), and Oyanagui (1984). Catalase activity was measured by following the decomposition of hydrogen peroxide (H_2O_2) (Abebi 1974). In this procedure, the decomposition of H_2O_2 was followed directly by a reduction in extinction at 240 nm. The difference in extinction (ΔE_{240}) per unit time was used as a measure of the catalase activity. Glutathione peroxidase (GSH-Px) activity was determined via the calorimetry of 2-nitro-5-thiobenzoic acid, a compound produced through a reaction between glutathione and 5,5'-dithiobis (2-nitrobenzoic acid) (Hafeman et al 1974). Protein contents were determined via the method of Lowry et al (1951), using bovine serum as a standard.

Statistics

Data are presented as the mean±SE of 6 determinations. Differences among groups were analyzed by Dunnett's test. The significance level was set at $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS

The levels of urea nitrogen and guanidino compounds in the serum are shown in Table 1. The amount of urea nitrogen and guanidino compounds were higher in the 2% arginine treated-groups than in the normal group. The blood urea nitrogen of normal rats was measured at 17.5 mg/dL, the level in the control group administered an 2% arginine diet was 21.6 mg/dL, corresponding to a 23% increase. A reduction of urea nitrogen in serum was clearly observed in the green tea tannin treated-groups, particularly at a dosage of 100 mg of body weight/day ($p<0.001$). A similar tendency was also found in Cr, as the serum Cr level treated with 50 mg of green tea tannin was markedly reduced as compared with the normal group. The levels of Arg and GAA in control rat were increased by 90.6% and 23.8%

than in the normal group. Moreover, the levels of both Arg and GAA were high in the rats treated with green tea tannin at a dosage of 100 mg.

Next, the levels of guanidino compounds were determined in urine. As is shown in Table 2, the levels of Arg, GAA, Cr, MG, and GSA were abundant in the control rats as compared with normal rats, having increased by 2.9 times in Arg, 73.4% in GAA, 42% in Cr, 39% in MG, and 4.5% in GSA. The levels of GAA, Cr, and GSA were reduced in the rats administered 100 mg of green tea tannin as compared with the control group, and a marked reduction of Cr was reached by level of normal group. However, the values of Arg and MG were not altered via the addition of green tea tannin.

The levels of Arg, GAA, Cr, and GSA in the kidney sample are shown in Table 3. The levels of these guanidino compounds in the rats fed on 2% arginine were increased above the levels observed in the normal rats: Arg increased by 15.5%, GAA by 14.7%, Cr by 20.3%, and GSA by 21.7%. GSA and GAA levels in the kidney were decreased as the result of the administration of 50 mg of green tea tannin. Although the levels of Cr and Arg increased in the rats fed 50 mg of green tea tannin as compared to the control group, this was not a significant difference.

Fig. 1 shows the urinary NO_2^- and NO_3^- values. The amount of excreted NO_3^- was 4.579 μmol/day in the normal group and 6.197 μmol/day in the control group. Thus, it was noted that the excretion of NO_3^- increased by 35.3% in the arginine treated-group as compared with the normal group.

Table 1. The effect of green tea tannin on guanidino compounds in the serum of rats fed on an arginine diet

Group	Dose (mg/kg BW/day)	Urea nitrogen (mg/dL)	Arg (mg/dL)	GAA (mg/dL)	Cr (mg/dL)
Normal rats		17.5±2.9	2.99±0.08	166.1±4.8	0.30±0.01
2% Arginine fed-rats					
Control		21.6±0.6^c	5.70±0.36^c	205.7±5.2^c	0.39±0.02^b
Green tea tannin	50	20.1±0.6^c	5.12±0.39^c	206.4±5.1^c	0.38±0.03^a
Green tea tannin	100	18.5±0.7^e	6.26±0.27^c	219.7±7.2^{c,d}	0.29±0.04^e

Statistical significance: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* normal values, $^d p<0.01$, $^e p<0.001$ *vs.* control values.
BW: Body weight, Arg: Arginine, GAA: Guanidinoacetic acid, Cr: Creatinine.
Normal: Rat group fed without 2% arginine.
Control: Rat group fed only 2% arginine without green tea tannin.

Table 2. The effect of green tea tannin on guanidino compounds in the urine of rats fed on an arginine diet

Group	Dose (mg/kg BW/day)	Arg (mg/rat/day)	GAA (μg/rat/day)	Cr (mg/rat/day)	MG (μg/rat/day)	GSA (μg/rat/day)
Normal rats		0.32±0.01	425±45.1	7.14±0.48	7.7±1.4	139.1±14.3
2% Arginine fed-rats						
Control		0.92±0.16^c	738.0±28.3^c	10.14±1.77^b	10.7±1.1^a	145.3±19.0
Green tea tannin	50	0.80±0.12^b	783.5±58.0^c	8.41±0.55	11.4±1.1^a	145.2±16.2
Green tea tannin	100	0.91±0.15^c	632.8±104.0^b	7.15±0.72^d	11.3±1.9^a	136.9±12.5

Statistical significance: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* normal values, $^d p<0.01$ *vs.* control values.
BW: Body weight, Arg: Arginine, GAA: Guanidinoacetic acid, Cr: Creatinine, MG: Methylguanidine, GSA: Guanidinosuccinic acid.
Normal: Rat group fed without 2% arginine.
Control: Rat group fed only 2% arginine without green tea tannin.

Table 3. The effect of green tea tannin on guanidino compounds in the kidney of rats fed on an arginine diet

Group	Dose (mg/kg BW/day)	Arg (μg/g kidney)	GAA (μg/g kidney)	Cr (μg/g kidney)	GSA (μg/g kidney)
Normal rats		227.45±15.52	59.85±2.82	45.41±9.51	42.96±5.12
2% Arginine fed-rats					
Control		262.74±5.78^b	68.67±2.67^b	54.61±1.64^b	52.30±3.19^a
Green tea tannin	50	263.89±6.53^b	66.50±2.17^a	57.91±2.97^c	50.75±5.29
Green tea tannin	100	293.65±15.66^{c,e}	67.86±2.59^b	61.53±2.49^{c,d}	53.89±1.91^b

Statistical significance: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ *vs.* normal values, $^d p<0.05$, $^e p<0.01$ *vs.* control values.
BW: Body weight, Arg: Arginine, GAA: Guanidinoacetic acid, Cr: Creatinine, GSA: Guanidinosuccinic acid.
Normal: Rat group fed without 2% arginine.
Control: Rat group fed only 2% arginine without green tea tannin.

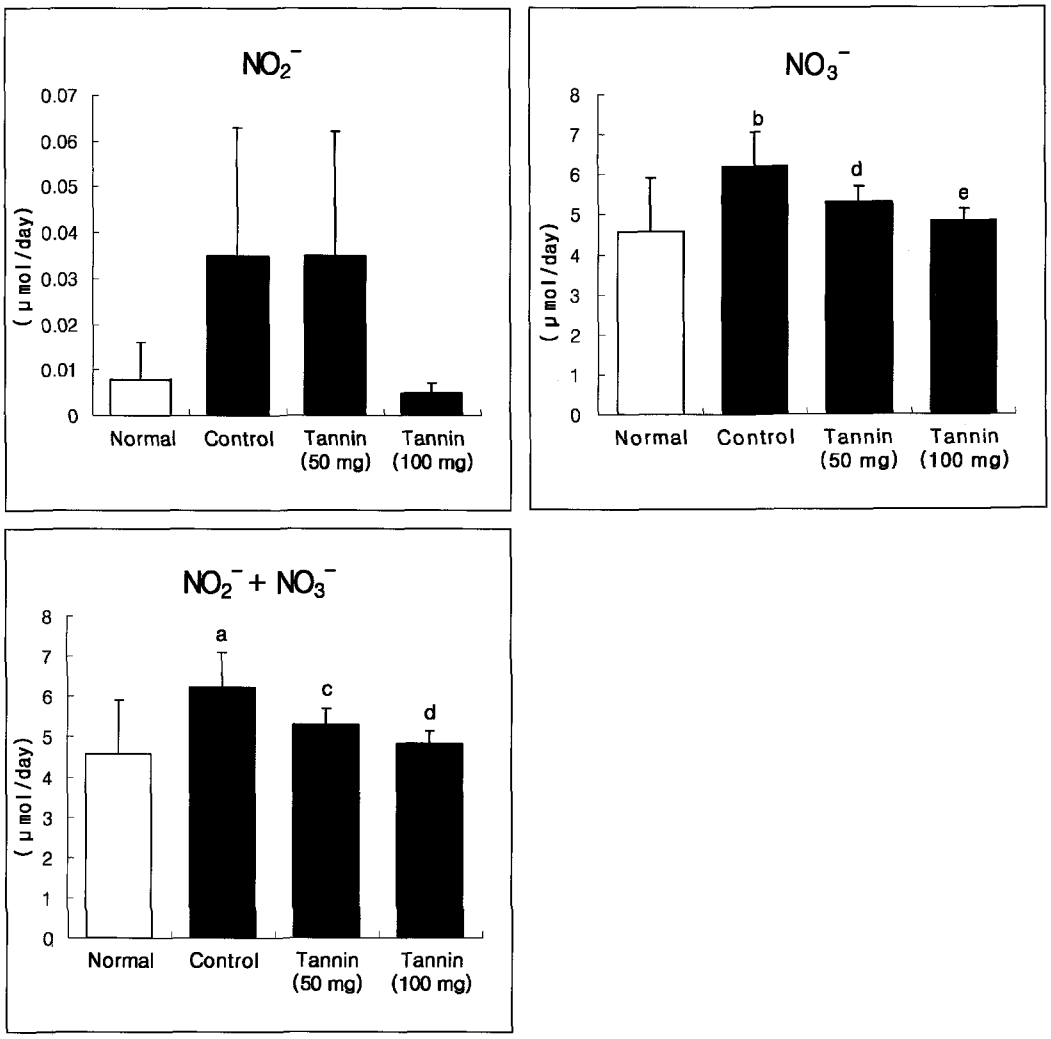

Fig. 1. The effect of green tea tannin on urinary NO_2^- and NO_3^- in rats fed on an arginine diets. Statistical significance: $^a p<0.01$, $^b p<0.001$ *vs.* normal values, $^c p<0.05$, $^d p<0.01$, $^e p<0.001$ *vs.* control values. Normal: Rat group fed without 2% arginine. Control: Rat group fed only 2% arginine without green tea tannin. 50 mg: 50 mg/kg body weight/day, 100 mg: 100 mg/kg body weight/day.

However, the level of urinary NO_3^- was significantly reduced in the green tea tannin treatment group ($p<0.001$, in 100 mg/kg body weight/day). A similar trend was observed in the urinary level of NO_2^- plus NO_3^-. The increase in the urinary excretion of NO_2^- plus NO_3^- by arginine treatment was diminished as the result of the administration of green tea tannin.

The results of the urinalysis are summarized in Fig. 2. In arginine fed-rats, the levels of urinary excretion of urea, protein, and glucose were also increased, by 76, 29, and 69%, respectively, as compared with the levels noted in normal rats. The administration of green tea tannin was determined to significantly reduce the excretion of these urinary constituents. Levels of excreted urea were measured at between 593.6 to 372.2 mg/dL (a 37% change, $p<0.01$); protein from 19.2 to 16.2 mg/day (a 16% change, $p<0.05$); and glucose from 5.63 to 3.57 mg/day (a 37% reduction, $p<0.001$) in rats administered a dosage of 100 mg of green tea tannin.

Fig. 3 shows the activity of reactive oxygen species-scavenging enzymes in the kidney. In comparison with normal rats, the rats administered an arginine diet evidenced a 32% reduction in SOD and a 57% reduction in catalase activities, whereas GSH-Px activity was increased briefly in the control group. The activities of both SOD and catalase in arginine-fed rats, however, increased when they were administered 50 mg and 100 mg of green tea tannin. SOD activity increased significantly from 17.69 to 25.04 U/mg protein in rats treated with 50 mg of green tea tannin. A similar change in catalase activity after the administration of 50 mg and 100 mg of green tea tannin was also observed, increasing from 76.0 to 111.6 and 114.9 U/mg of protein, respectively. No particular variation in GSH-Px activity was noted following the administration of green tea tannin.

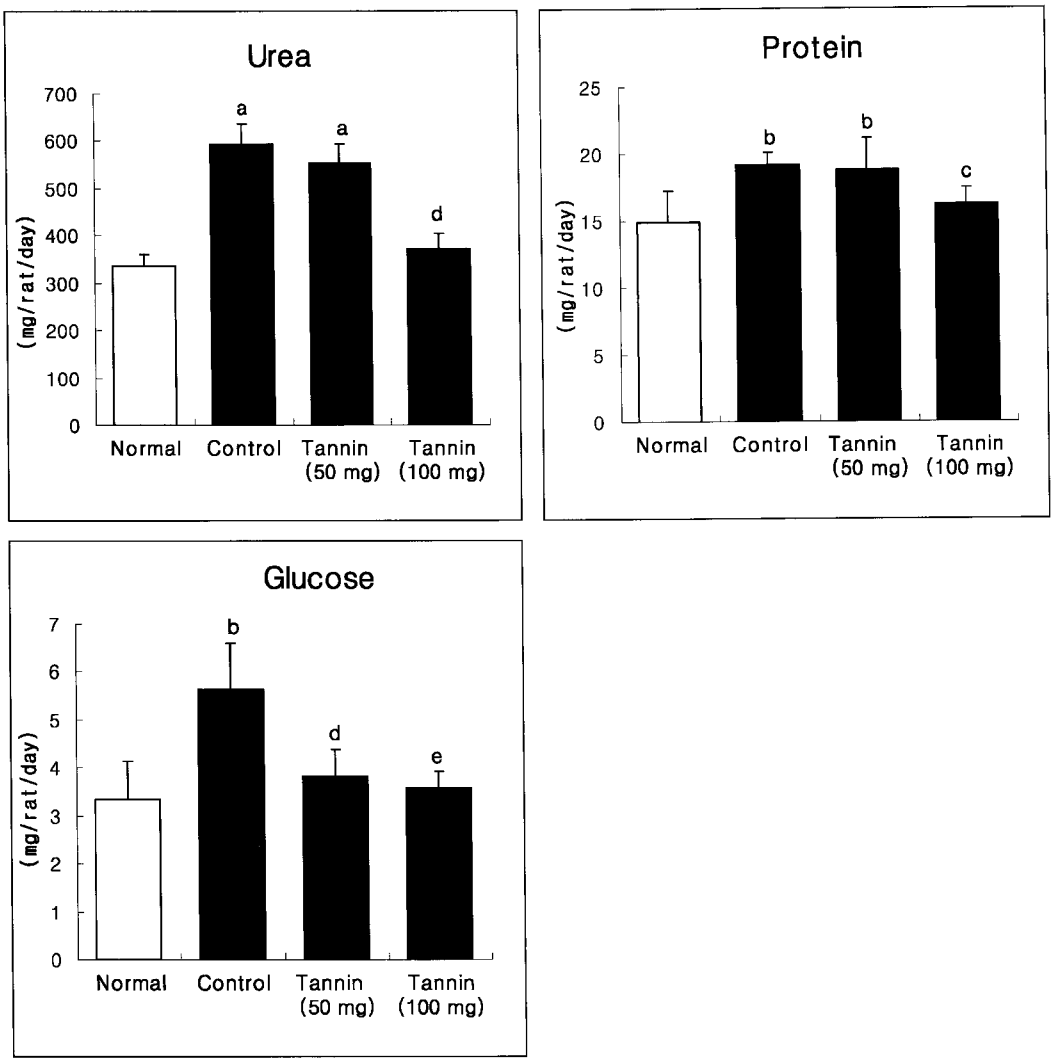

Fig. 2. The effect of green tea tannin on urinary constituents in rats fed on an arginine diet. Statistical significance: $^{a}p<0.05$, $^{b}p<0.01$ *vs.* normal values, $^{c}p<0.05$, $^{d}p<0.01$, $^{e}p<0.001$ *vs.* control values. Normal: Rat group fed without 2% arginine. Control: Rat group fed only 2% arginine without green tea tannin. 50 mg: 50 mg/kg body weight/day, 100 mg: 100 mg/kg body weight/day.

DISCUSSION

Arginine is synthesized from citrulline primarily in the liver and kidney. Arginine is metabolized either in ornithine and urea, primarily in the liver and intestine, or in citrulline and nitric oxide in a large number of cell types. In the kidney, citrulline is transformed into arginine and is subsequently released to the peripheral tissues (Cynober et al 1995). Adult mammals, including human beings, can fulfill all of their arginine needs via endogenic synthesis, whereas immature mammals and disease states require dietary arginine. Arginine, which is employed in the synthesis of body proteins, is essential for ammonia detoxification via urea synthesis. It is a precursor for polyamine synthesis and is the source for the formation of creatine, a major source of high energy phosphate for ATP regeneration in the muscle. At supraphysiologic doses, arginine is utilized for hormones that control growth and metabolism (Visek 1985; Visek 1986). However, it has been reported that excessive doses of arginine induce severe pancreatic lesions and oxygen-derived free radicals are generated at an early stage of arginine-induced acute pancreatitis (Czako et al 1998; Mizunma et al 1984; Varga et al 1997).

Arginine is a source of NO, which functions as an endothelium-derived relaxing factor and neuronal messenger. By way of contrast, NO may perform a function in hypoxia and reoxygenation injuries, owing to its free radical nature and high reactivity with O_2^- to yield $ONOO^-$, an oxidant molecule (Yu et al 1994). The final products of NO *in vivo* are NO_2^- and NO_3^-. The relative proportion of the two compounds cannot be predicted with certainty. Therefore, the urinary excretion of both NO_2^- plus NO_3^- is determined

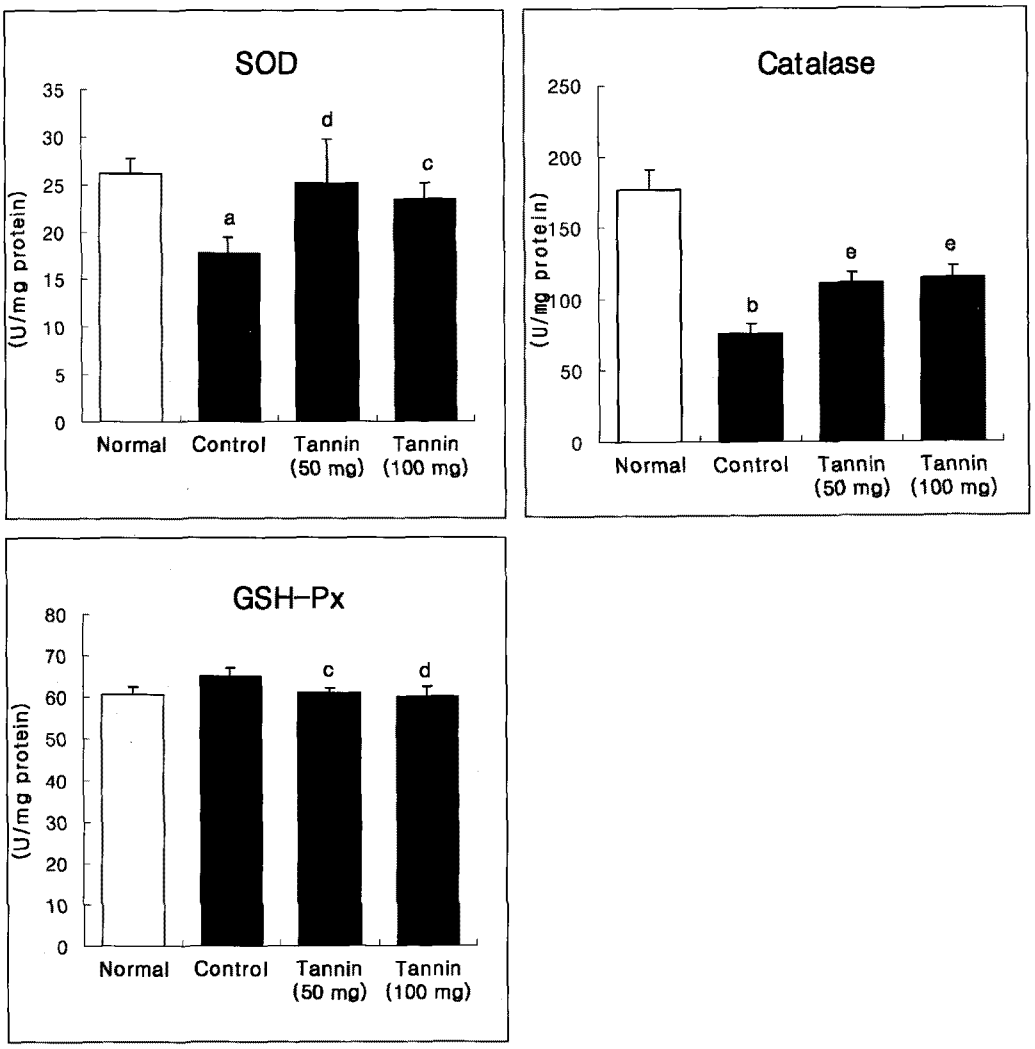

Fig. 3. The effect of green tea tannin on oxygen species-scavenging enzymes in kidney of rats fed on an arginine diet. Statistical significance: $^{a}p<0.01$, $^{b}p<0.001$ *vs.* normal values, $^{c}p<0.05$, $^{d}p<0.01$, $^{e}p<0.001$ *vs.* control values. Normal: Rat group fed without 2% arginine. Control: Rat group fed only 2% arginine without green tea tannin. 50 mg: 50 mg/kg body weight/day, 100 mg: 100 mg/kg body weight/day.

as an index of NO production (Sierra et al 1998). As compared with the arginine nontreated-group in the present study, the arginine treated-groups evidenced higher urinary excretion of $NO_2^-+NO_3^-$. It has been proposed that the increase of NO production may be attributable to dietary arginine. These increases in urinary NO excretion were ameliorated by the addition of green tea tannin, particularly by green tea tannin at 100 mg/kg of body weight/day. Thus, we conclude that the production of NO is depressed due to green tea tannin treatment.

The metabolism of arginine is regulated by enzymes such as glycine amidinotransferase, arginase, and NOS. Arginine is converted to GAA by glycine amidinotransferase in the kidney, and subsequently to creatine in the liver. Creatine is converted to creatinine via creatinine phosphate and excreted into the urine. Creatinine is metabolized further to MG (Yokozawa et al 1991A). Natelson et al (1979) proposed that the guanidino carbon of GSA originated from urea, and that urea cycle enzymes catalyzed the reactions for GSA production. Among these guanidino compounds, Cr, MG and GSA are known to accumulate in the blood with the progression of renal failure as uremic toxins (Cohen 1970; Giovannetti et al 1968; Yokozawa et al 1986). In addition, reactive oxygen species such as ·OH and O_2^- are important for Cr, MG and GSA synthesis (Aoyagi et al 1996A; Aoyagi et al 1996B; Fujitsuka et al 1994). In the present study, treatment with 2% arginine resulted in a significant increase in the levels of guanidino compounds in the serum, urine, and kidneys. The serum levels of arginine, GAA, and Cr were increased in the 2% arginine fed-rats, and also increased the quantity of urea nitrogen. By way of contrast, urea nitrogen and Cr levels were reduced by the

administration of green tea tannin. It is known that GSA formation increased depending on the amount of urinary urea and the serum urea levels (Aoyagi et al 1983; Cohen and Patel 1982; Kopple et al 1968). Thus, the decrease of blood urea nitrogen due to treatment with green tea tannin shows that GSA formulation is reduced. It has been reported that the blood levels of urea nitrogen, MG, Cr, and GSA were reduced significantly in rats administered (-)-epigallocatechin 3-*O*-gallate, thereby indicating that the elimination of free radicals results in an alleviation of renal disorder (Yokozawa et al 1986; Yokozawa et al 1991B). The urinary excretion of guanidino compounds increased with arginine treatment; in particular, the quantity of arginine was 2.9 times higher than that in normal rats, and the level of urinary Cr and MG also increased by a factor of 1.4 in rats fed on 2% arginine. Orita et al (1978) reported that arginine administration resulted in a marked increase in urinary MG excretion both in the uremic rats and patients. In the present study, the administration of green tea tannin reduced the excretion of urinary Cr, whereas the level of MG was unaltered. In the case of urea, protein, and glucose in the urine, the increment after arginine treatment was reduced significantly by the administration of green tea tannin; the improvement in glucose levels was particularly remarkable ($p<0.001$). Therefore, we conclude that arginine administered to rats is metabolized to guanidino compounds, and these substances accumulate in the serum, urine, and kidneys.

In this study, we measured the endogenous scavengers SOD, catalase, and GSH-Px in the kidney, in order to elucidate the participation of free radicals in the process of Arg-induced renal failure. 2% arginine influenced the activity of radical-scavenger enzymes in the kidney, resulting in a reduction in the activity of SOD, an enzyme that catalyzes the disproportionation of O_2^- into H_2O_2. Moreover, arginine induced an reduction in the activity of catalase, an enzyme that specifically eliminates H_2O_2. On the other hand, We noted no change in GSH-Px, an enzyme that helps to eliminate H_2O and is localized in the matrix of the mitochondria. These results suggest that arginine influences peroxisomes in the kidney. Additionally, the lowered SOD and catalase activities imply the generation of oxygen-derived free radicals, following renal failure. However, the treatment of green tea tannin was shown to increase the activity of SOD and catalase, both of which were reduced by arginine treatment. Yokozawa et al (1996) previously reported that treatment with (-)-epigallocatechine 3-*O*-gallate, the main ingredient of green tea, increased the activity of SOD and catalase in nephrectomized rats. Additionally, it was reported that a green tea tannin mixture exerted a potent scavenger effect using cultured cells, and also exerted an inhibitory effect on oxidative stress-induced apoptosis (Yokozawa et al 1998). In conclusion, it is suggested that green tea tannin treatment may prevent the generation of reactive oxygen metabolites.

In the current study, the administration of excessive arginine to rats increased the formation of urea, guanidino compounds, and NO, and also increased the excretion of urinary protein and glucose. In addition, oxygen species-scavenging enzymes in the kidney were reduced by arginine treatment. These results demonstrate that green tea tannin may exert a protective effect on severe renal injuries induced by excessive arginine.

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