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Influence of Green Tea Polyphenol in Rats with Arginine-Induced Renal Failure

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To determine whether green tea polyphenol ameliorates the pathological conditions induced by excessive dietary arginine, green tea polyphenol was administered to rats at a daily dose of 50 or 100 mg/kg body weight for 30 days with a 2% w/w arginine diet. In arginine-fed control rats, urinary and/or serum levels of guanidino compounds, nitric oxide (NO), urea, protein, and glucose increased significantly, while the renal activities of the oxygen species-scavenging enzymes superoxide dismutase (SOD) and catalase decreased, compared with casein-fed rats. However, rats given green tea polyphenol showed significant and dose-dependent decreases in serum levels of creatinine (Cr) and urea nitrogen and urinary excretion of Cr, and they exerted a slight reduction of nitrite plus nitrate, indicating that green tea polyphenol reduced the production of uremic toxins and NO. In addition, in arginine-fed rats the urinary urea, protein, and glucose level increases were reversed by the administration of green tea polyphenol. Moreover, in rats given green tea polyphenol the SOD and catalase activities suppressed by excessive arginine administration increased dose-dependently, implying the biological defense system was augmented as a result of free radical scavenging. These results suggest that green tea polyphenol would ameliorate renal failure induced by excessive dietary arginine by decreasing uremic toxin, and NO production and increasing radical-scavenging enzyme activity.

KEYWORDS: Green tea; polyphenol; arginine; guanidino compounds; nitric oxide; superoxide dismutase; catalase; renal failure

INTRODUCTION

Arginine is used in the synthesis of body proteins and it is a crucial vehicle for transport, storage, and excretion of nitrogen. In particular, arginine is essential for ammonia detoxification via urea synthesis, which prevents metabolic derangements caused by elevated tissue ammonia levels. Arginine is also used in the synthesis of polyamines that play a key role in cell division, tissue growth, and differentiation (*1*). Therefore, the physiological requirement of tissues and organs for arginine should be supplied by endogenous synthesis and the diet. However, administration of excess arginine causes imbalance of amino acids and changes in protein metabolism. In addition, arginine is the key substance of guanidino compounds such as creatinine (Cr), methylguanidine (MG), and guanidinosuccinic acid (GSA), which are considered to be uremic toxins (*2*, *3*). Moreover, nitric oxide (NO), an important mediator of diverse pathological damage because of its toxic effects, is formed from arginine by the NO synthase family of enzymes. Numerous uremic toxins such as Cr and MG as well as NO produced from excessive arginine are responsible for acute renal failure (*4*−*7*). Furthermore, it was found that excessive administration of arginine induced pancreatitis in rats and enhanced hypoxia/reoxygenation injury (*8*−*10*). These reports suggest that, although it is an important amino acid in the body, excessive doses of arginine can be pathogenic.

Recently, attention has been focused on the pharmaceutical effects of polyphenols, typical constituents of tea, fruit, and vegetables, owing to their antioxidative activity. In particular, green tea is a widely consumed beverage and contains mainly flavan-3-ol polyphenols, known to be excellent antioxidants that directly scavenge free radicals and protect against pathological damage such as hypertension, tumorigenesis, and renal diseases (*11*−*15*). On the basis of this evidence, the effect of green tea polyphenol was investigated in arginine-fed rats, a useful experimental model of renal failure resulting from uremic toxins and NO synthesis caused by excessive dietary arginine.

MATERIALS AND METHODS

Green Tea Polyphenol. The green tea polyphenol mixture employed was Sunphenon (Taiyo Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea with a recovery rate of about 9.6%, by weight, of the original pulverized Japanese green tea, as reported previously (*16*). It was mainly composed of, by weight, (−)-epigallocatechin 3-*O*-gallate (18.0%), (−)-gallocatechin 3-*O*-gallate (11.6%), (−)-epicatechin 3-*O*-gallate (4.6%), (−)-epigallocatechin

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Table 1. Effect of Green Tea Polyphenol on Guanidino Compounds and Urea Nitrogen in Serum

group	dose (mg/kg B. W./day)	arginine (mg/dL)	GAA (μg/dL)	Cr (mg/dL)	urea nitrogen (mg/dL)
casein-fed rats		2.99 ± 0.08	166 ± 5	0.30 ± 0.01	17.5 ± 2.9
arginine-fed rats					
control		5.70 ± 0.36^{b}	206 ± 5^{b}	0.39 ± 0.02^{b}	21.6 ± 0.6^{a}
polyphenol	50	5.26 ± 0.27^{b}	206 ± 5^{b}	0.38 ± 0.03^{b}	20.1 ± 0.6
polyphenol	100	$5.12 \pm 0.39^{b,c}$	$220 \pm 7^{b,d}$	0.29 ± 0.04^{e}	18.5 ± 0.7^{c}

Statistical significance: $^{a}p < 0.01$, $^{b}p < 0.001$ vs casein-fed rats; $^{c}p < 0.05$, $^{d}p < 0.01$, $^{e}p < 0.001$ vs arginine-fed control rats.

Table 2. Effect of Green Tea Polyphenol on Guanidino Compounds in Urine

group	dose (mg/kg B. W./day)	arginine (mg/day)	GAA (μg/day)	Cr (mg/day)	MG (μg/day)
casein-fed rats		0.32 ± 0.01	426 ± 45	7.14 ± 0.48	7.7 ± 1.4
arginine-fed rats					
control		0.92 ± 0.16^{c}	633 ± 104^{c}	10.14 ± 1.77^{c}	10.7 ± 1.1^{a}
polyphenol	50	0.91 ± 0.15^{c}	738 ± 28^{c}	8.41 ± 0.55	11.4 ± 1.1^{b}
polyphenol	100	0.80 ± 0.12^{c}	$784 \pm 58^{c,d}$	7.15 ± 0.72^{e}	11.3 ± 1.9^{b}

Statistical significance: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs casein-fed control rats; $^{d}p < 0.01$, $^{e}p < 0.001$ vs arginine-fed control rats.

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

Animals and Treatment. *The Guiding Principles for the Care and Use of Laboratory Animals and Guidelines for Animal Experimentation* approved by the Japan Pharmacological Society and Japanese Association for Laboratory Animal Science, respectively, were followed in these experiments. Male Wistar rats with a body weight of 120−130 g were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 25 °C) and humidity (about 60%) were controlled automatically. During several days of adaptation, they were fed on an 18% casein diet consisting of (per 100 g): casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture 4 g, vitamin mixture 1 g, cellulose powder 2 g and choline chloride 0.1 g. Following the adaptation period, the animals were divided into 4 groups (n=6/group), avoiding any intergroup difference in body weight gain: one group was fed on the 18% casein diet (casein diet) and the others were pair-fed the 18% casein diet containing 2% w/w arginine (2% arginine diet, dosage of arginine: 400 mg (kg of body weight)$^{-1}$ day^{-1}). The administration dose of arginine was determined on the basis of other reports (*9*, *10*) and the productions of uremic toxins and NO by arginine. Throughout the arginine feeding period, the control group was given water, while the other two groups were given green tea polyphenol dissolved in water orally at a dose of 50 or 100 mg (kg of body weight)$^{-1}$ day^{-1} for 30 days using a stomach tube. Urine specimens were collected for 1−2 days before sacrifice. After sacrifice, blood samples were obtained by cardiac puncture and the serum was separated immediately by centrifugation. The kidneys were perfused through the renal artery with ice-cold physiological saline, extirpated and frozen at −80 °C until assay.

Analysis of Serum and Urine Samples. To determine the levels of guanidino compounds (arginine, guanidinoacetic acid (GAA), Cr, and MG), the serum and urine samples were deproteinized by adding trichloroacetic acid (final concentration 10% v/v). The supernatant obtained by centrifugation at 3000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system, according to the method of Higashidate et al. (*17*). A fluorescence spectrometer, model FP-210 (excitation 365 nm, emission 495 nm; Japan Spectroscopic Co., Tokyo, Japan) was used to detect the guanidino compounds on the column. Urea nitrogen concentrations were determined using the commercial reagent BUN Kainos (Kainos Laboratories, Tokyo, Japan) and nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were measured with a NOX measuring device, ENO-10 (Eicom Co. Ltd., Tokyo, Japan). Urea, protein, and glucose concentrations were assayed by the Archibald (*18*), sulfosalicylic acid (*19*), and Momose (*20*) methods, respectively.

Enzyme Assays. Superoxide dismutase (SOD) activity was determined according to the nitrous acid method described by Elstner and Heupel (*21*) and Oyanagui (*22*), which is based on the inhibition of NO_2^- formation by hydroxylamine in the presence of superoxide (O_2^-) generators. Catalase activity was evaluated by following the decomposition of hydrogen peroxide (H_2O_2) directly by monitoring the decrease in extinction at 240 nm (*23*). Glutathione peroxidase (GSH−Px) activity was measured by a colorimetric assay that determines the concentration of 2-nitro-5-thiobenzoic acid, a compound produced by the reaction between glutathione (GSH) and 5,5′-dithiobis (2-nitrobenzoic acid) (*24*). Protein levels were determined by the micro-biuret method with bovine serum albumin as the standard (*25*).

Statistics. Data are presented as means ±SE of six determinations. Differences among groups were analyzed by Dunnett's test and those at $p < 0.05$ were accepted as significant.

RESULTS

Serum. Table 1 shows the effects of green tea polyphenol on the serum guanidino compound and urea nitrogen levels of arginine-fed rats. The serum arginine, GAA, Cr, and urea nitrogen levels of arginine-fed rats were significantly higher than those of casein-fed rats. The rats given green tea polyphenol at a dose of 100 mg showed significantly lower level of arginine compared with control rats. Oral administration of green tea polyphenol reduced the Cr and urea nitrogen levels significantly and dose-dependently, but increased the GAA level. By the administration of green tea polyphenol at a daily dose of 100 mg/kg of body weight, the concentration of Cr was reduced by 26% and that of urea nitrogen was reduced by 14%.

Urine. Table 2 shows the urinary excretion results for the guanidino compounds. The administration of the 2% arginine diet increased the urinary levels of arginine, GAA, Cr and MG by 2.88-, 1.49-, 1.42-, and 1.39-fold, respectively. In contrast, the urinary excretion of Cr in arginine-fed control rats decreased significantly by 29% in rats given 100 mg (kg of body weight)$^{-1}$ day^{-1} of green tea polyphenol, almost reaching the casein-fed level. A similar trend was found for arginine excretion, although the magnitude of the decrease was less than that for Cr. The urinary excretion rate of arginine decreased by 13% in rats given green tea polyphenol 100 mg (kg body weight)$^{-1}$ day^{-1} compared with arginine-fed control rats. The urinary excretions of GAA and MG increased in green tea polyphenol-treated rats.

Table 3. Effect of Green Tea Polyphenol on NO Metabolites in Urine

group	dose (mg/kg B. W./day)	NO_2^- (μmol/day)	NO_3^- (μmol/day)	$NO_2^- + NO_3^-$ (μmol/day)
casein-fed rats		0.008 ± 0.008	4.579 ± 1.334	4.587 ± 1.342
arginine-fed rats				
control		0.035 ± 0.028	6.197 ± 0.857[a]	6.232 ± 0.881[a]
polyphenol	50	0.035 ± 0.027	5.277 ± 0.417	5.312 ± 0.410
polyphenol	100	0.030 ± 0.010	4.821 ± 0.328	4.851 ± 0.328

Statistical significance: [a] p <0.05 vs casein-fed rats.

Table 4. Effect of Green Tea Polyphenol on Urinary Chemical Components

group	dose (mg/kg B. W./day)	urea (mg/day)	protein (mg/day)	glucose (mg/day)
casein-fed rats		271 ± 47	14.9 ± 2.4	3.34 ± 0.79
arginine-fed rats				
control		505 ± 32[c]	19.2 ± 0.9[b]	5.63 ± 0.98[c]
polyphenol	50	502 ± 49[c]	18.7 ± 2.4[a]	3.81 ± 0.57[d]
polyphenol	100	385 ± 34[b,e]	16.2 ± 1.2	3.57 ± 0.37[e]

Statistical significance: [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 vs casein-fed rats; [d] p < 0.01, [e] p < 0.001 vs arginine-fed control rats.

Table 5. Effect of Green Tea Polyphenol on Oxygen Species-Scavenging Enzymes in Kidney

group	dose (mg/kg B. W./day)	SOD (U/mg of protein)	catalase (U/mg of protein)	GSH-Px (U/mg of protein)
casein-fed rats		26.09 ± 1.66	177.0 ± 13.5	60.61 ± 1.99
arginine-fed rats				
control		17.69 ± 1.68[b]	76.0 ± 7.0[b]	64.76 ± 2.18[a]
polyphenol	50	23.32 ± 1.75[c]	111.6 ± 6.6[b,e]	60.94 ± 1.24[c]
polyphenol	100	25.04 ± 4.68[d]	114.9 ± 7.6[b,e]	60.04 ± 2.55[d]

Statistical significance: [a] p < 0.05, [b] p < 0.001 vs casein-fed rats; [c] p < 0.05, [d] p < 0.01, [e] p < 0.001 vs arginine-fed control rats.

Table 3 represents the effect of green tea polyphenol on urinary NO metabolite levels. In comparison with the casein-fed group, the 2% arginine-fed group showed an obvious increase in the level of the NO metabolites NO_2^- plus NO_3^-. However, administration of green tea polyphenol reduced the NO_2^- plus NO_3^- level slightly in a dose dependent manner although the significance was not shown. The urinary NO_2^- plus NO_3^- level was reduced by 15 and 22% by 50 and 100 mg (kg of body weight)$^{-1}$ day^{-1} green tea polyphenol, respectively. In arginine-fed rats, urinary excretion of urea, protein, and glucose increased by 1.86-, 1.29-, and 1.69-fold, respectively, compared with casein-fed rats (**Table 4**). Administration of green tea polyphenol at a dose of 100 mg (kg body weight)$^{-1}$ day^{-1} led to a 24% decrease in the excretion of urea. Similarly, the excretion of protein showed a significant decrease at the 100 mg (kg body weight)$^{-1}$ day^{-1} dosage by 16%. In addition, the excretion of glucose resulted in 32 and 37% decreases after administration of the 50 and 100 mg (kg of body weight)$^{-1}$ day^{-1} dosages, respectively.

Kidney. Table 5 shows the activities of the reactive oxygen species-scavenging enzymes SOD, catalase and GSH−Px in the kidney. After the administration of arginine, the activity of SOD declined by 32% and that of catalase did by 57%. Rats given green tea polyphenol orally at both the 50 and 100 mg (kg of body weight)$^{-1}$ day^{-1} dosage levels showed respective increases in SOD (by 32 and 42%) and catalase (by 47 and 51%) activities compared with the corresponding control values. In contrast, GSH−Px activity was lower in the green tea polyphenol-treated rats than in the control rats, whereas it was not significantly different in the casein-fed and green tea polyphenol-treated rats.

DISCUSSION

Arginine supplies a structure that carries nitrogen in the urea cycle, which serves to generate guanidino compounds (*2, 3, 26*). Cleavage of arginine results in the formation of Cr, MG, GSA, and other guanidino compounds, which are well-known as uremic toxins (*3*). Arginine is converted to GAA by glycine amidinotransferase in the kidney and then to creatine in the liver. Creatine is converted to Cr via creatinine phosphate and excreted in the urine and Cr is metabolized further to MG (*27*).

The blood concentrations and urinary excretion of the uremic toxins Cr, MG, and GSA increase with the progression of renal failure (*28*−*30*). In addition, the elevated urea concentration can serve as an indicator of renal dysfunction and, in patients with acute renal failure, the correlation between the severity of the pathological condition and the concentration of blood urea nitrogen is actually relatively good. In our present study, feeding rats arginine enhanced the levels of urea in the serum and urine, which implies that renal function of which toxic substances were eliminated when their concentrations in the body fluids rose was impaired. Our study also demonstrated that the serum and urinary levels of arginine, GAA, Cr and MG were increased by the 2% arginine diet, implying renal failure was caused by the uremic toxins. However, green tea polyphenol reduced the levels of urea and Cr in the serum and urine; therefore, it would be expected to ameliorate renal failure by reducing the levels of uremic toxins. It is known that GSA formation increases depending on the urinary and serum urea levels (*31*−*33*). Thus, the decreases in the blood and urinary urea nitrogen levels resulting from green tea polyphenol administration imply that GSA formation also would be lowered. A previous study demonstrated that blood levels of urea nitrogen, MG, Cr, and

GSA decreased significantly in rats with adenine-induced renal failure given (−)-epigallocatechin 3-*O*-gallate, the main polyphenol of green tea, suggesting that elimination of uremic toxins leads to relief of renal disorders (*34*). Furthermore, the magnitudes of the elevations in the urinary urea, protein and glucose levels due to excessive dietary arginine were reduced significantly by administration of green tea polyphenol, which suggests that green tea polyphenol would improve the impaired metabolism caused by excessive arginine. These results provide evidence that arginine administered to rats is metabolized to guanidino compounds, which accumulate in the serum and urine, leading to renal impairment. In contrast, green tea polyphenol appears to improve the renal disorder caused by excessive dietary arginine through reducing the levels of uremic toxins.

The guanidino nitrogen of arginine undergoes oxidation by NO synthases to yield NO. NO plays a considerable role in hypoxia and reoxygenation injury due to its free radical nature and high reactivity with O_2^- to form peroxynitrite, a contributor to tissue injury in several diseases, including renal failure. The final products of NO in vivo are NO_2^- and NO_3^-. Therefore, urinary excretion of NO_2^- plus NO_3^- is determined as an index of NO production, and the levels of products of the arginine-NO pathway, such as NO_2^- and NO_3^-, in biological fluids can be used as clinical markers for monitoring certain pathologic conditions and the progress of their treatment (*35*, *36*). Noris et al. (*37*) reported that arginine levels and NO synthesis are higher in uremics than healthy volunteers, suggesting an explanation for the increased NO synthesis in uremia. Moreover, it is well established that serum and urinary concentrations of NO_2^- and NO_3^- are elevated in patients with renal failure (*38*). As we also demonstrated in the present investigation, the level of NO_2^- plus NO_3^- is higher in arginine-fed rats than casein-fed rats. It is believed that the increase in NO production is attributable to dietary arginine and that it causes renal failure. Green tea polyphenol suppressed the production of NO, therefore, it would be expected to ameliorate the renal injury induced by excessive arginine.

In this study, we measured the activities of the radical-scavenging enzymes SOD, catalase and GSH−Px in the kidney to elucidate whether free radicals participate in the process of arginine-induced renal failure. It is well accepted that free radicals and decreased activities of these antioxidative enzymes are involved in various ways in the occurrence and progression of renal failure (*39*, *40*). Our results showed that the free radical-scavenging system was impaired in arginine-fed rats. The results about the antioxidative enzyme activities under oxidative stress are controversial. Consistent with our result, Gaertner et al. (*41*) demonstrated that reactive oxygen species are involved in the mesangioproliferative glomerulonephritis and despite glomerular oxidative stress, the activities of antioxidative enzymes, SOD, catalase and GSH−Px, even decreased during the course of disease. Arginine influenced the activities of radical-scavenging enzymes in the kidney, leading to a decrease in the activity of SOD, which catalyzes disproportionation of O_2^- to H_2O_2. Moreover, the activity of catalase, which specifically eliminates H_2O_2, was also suppressed in rats fed the 2% arginine diet. These results indicate that arginine affected the activity of antioxidative enzymes in the renal peroxisomes and the reductions in the SOD and catalase activities induced by arginine imply that oxygen-derived free radicals were generated and the biological defense system was weakened. However, the administration of green tea polyphenol increased the activities of SOD and catalase.

Reactive oxygen species, such as hydroxyl radicals and O_2^-, as well as uremic toxins are also mainly responsible for renal failure (*4*−*7*). In addition, free radicals play a considerable role in the synthesis of the uremic toxins Cr, MG and GSA (*42*−*44*). In light of these reports and our present results, green tea polyphenol would be expected to play a crucial role as a scavenger of free radicals and inhibitor of uremic toxin synthesis, thereby eventually ameliorating renal failure. Consistent with the results of our present investigation, in a previous study we demonstrated that treating nephrectomized rats with green tea polyphenol increased the activities of SOD and catalase (*45*). Furthermore, green tea polyphenol has also been reported to exert a potent scavenging effect through the inhibition of oxidative stress-induced apoptosis in a cell culture system (*34*).

Our results suggest that excessive dietary arginine evokes renal failure by increasing the production of uremic toxins and NO, and by decreasing oxygen species-scavenging enzyme activity in the kidney. However, green tea polyphenol showed a protective effect against the renal injury induced by arginine. Therefore, we expect that green tea polyphenol has the potential to be a promising new therapeutic approach to renal disorders.

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