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Green tea polyphenols and dietary fibre protect against kidney damage in rats with diabetic nephropathy

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Abstract

In this study we examined the effect of green tea polyphenols (GTP) and partially hydrolysed guar gum (PHGG) as dietary fibre on diabetic nephropathy, using rats that had been subjected to subtotal nephrectomy and injection of streptozotocin. The subtotally nephrectomized rats were subjected to resection of three-quarters of the kidney. Rats with diabetic nephropathy were divided into four groups: untreated controls, and animals that received GTP (100 mg kg^{-1} body weight day^{-1}), PHGG (100 mg kg^{-1} body weight day^{-1}) and GTP plus PHGG (50 mg kg^{-1} body weight day^{-1} plus 50 mg kg^{-1} body weight day^{-1}). After 50 days of administration, attenuation of urinary protein excretion and the morphological changes peculiar to diabetic nephropathy were observed in all three treated groups. Furthermore, the group treated with GTP plus PHGG showed an improvement of kidney weight and serum levels of urea nitrogen, creatinine and creatinine clearance. Hyperglycaemia, as assessed in terms of blood glucose and glycosylated protein levels, was also improved by administration of GTP plus PHGG. On the other hand, GTP administration increased the activity of superoxide dismutase in the kidney to a significant extent. A significant reduction in the total cholesterol concentration was also observed in the PHGG-treated group. These results suggest that GTP and PHGG could be beneficial as additional therapy in the management of diabetic nephropathy.

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Introduction

Diabetic nephropathy is one of the major problems affecting patients with diabetes. Once nephropathy has progressed to the stage where persistent proteinuria is present, the prognosis is poor and the invariable outcome is end-stage renal failure and dialysis therapy. To prevent and treat diabetic nephropathy, current methods using agents such as angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers and antihypertensive drugs have been tried in clinical practice (Parving et al 1983; Preston 1999; Brenner et al 2001). Despite these treatments, large numbers of patients still develop intractable diabetic nephropathy. The therapeutic interventions that might ameliorate the specific alterations in diabetic nephropathy therefore represent one of the most promising areas of research today.

The pathogenesis of diabetic nephropathy has been extensively discussed for many years, and it has been accepted that oxidative stress is closely involved as a causative factor stemming from persistent hyperglycaemia (Baynes & Thorpe 1999; Ha & Kim 1999). On the other hand, it is widely believed that lifestyle factors, including food intake and exercise, can significantly contribute to the prevention and therapy of diabetes and its complications. Recent evidence from epidemiological studies has shown that a fibre-deficient diet is correlated with an increased incidence of type 2 diabetes (Parillo & Riccardi 2004). In this connection, antioxidants and dietary fibre have received much attention as major functional components of foods that have the potential to prevent various diseases because of their distinctive biological activity and low toxicity.

Green tea, a beverage that is widely consumed worldwide, is known to have excellent antioxidant properties. Both in vitro and in vivo, green tea and polyphenols extracted from it have been shown to exhibit anti-hypertensive, anti-mutagenic and

anti-carcinogenic effects (Mukhtar et al 1992; Yang & Wang 1993; Hodgson et al 1999) because of their antioxidative activity. Furthermore, we have reported that green tea polyphenols (GTP) inhibited mesangial proliferation and significantly prolonged renal survival in experimental models of glomerulosclerosis, suggesting that their renoprotective effects are based on antioxidative activity (Yokozawa et al 1993, 1996). Guar gum, a water-soluble dietary fibre, and its enzymatic hydrolysate (partially hydrolysed guar gum, PHGG) have been shown to decrease levels of serum cholesterol and postprandial blood glucose (Bijlani 1985; Spiller 1994), and to support the growth of beneficial hindgut bacteria. It has therefore been hypothesized that these dietary factors might play an additional role in the treatment of diabetic nephropathy. In the present study, to explore this possibility, we used an animal model of diabetic nephropathy to examine the effects of GTP and PHGG, both separately and in combination.

Materials and Methods

Materials

The GTP mixture used in this study was Sunphenon (Taiyo Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea with a recovery rate of 9.6% by weight of the original pulverized Japanese green tea, as reported previously (Sakanaka et al 1989). It was composed mainly of (−)-epigallocatechin 3-*O*-gallate (18.0%), (−)-gallocatechin 3-*O*-gallate (11.6%), (−)-epicatechin 3-*O*-gallate (4.6%), (−)-epigallocatechin (15.0%), (+)-gallocatechin (14.8%), (−)-epicatechin (7.0%) and (+)-catechin (3.5%). PHGG was supplied by Taiyo Kagaku Co., Ltd., Japan (Yamamoto et al 1990).

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 (160–170 g) were obtained from Japan SLC Inc. (Hamamatsu, Japan). They were kept in wire-bottomed cages and exposed to a 12 h/12 h light/dark cycle. The room temperature (about 23°C) and humidity (about 60%) were controlled automatically. The rats were allowed access to laboratory pellet chow (Clea Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water ad libitum. According to the method reported previously (Yokozawa et al 2001), the rats underwent resection of half of the left kidney and then, 7 days later, total excision of the right kidney. Thereafter, they were injected intraperitoneally with 25 mg kg^{-1} body weight streptozotocin (STZ) dissolved in 10 mM citrate buffer (pH 4.5) following overnight fasting. Ten days after STZ injection, the glucose and urea nitrogen levels of blood taken from the tail vein of each rat were determined, and the rats were then divided into the following four groups, avoiding any intergroup differences in these blood indices: control group (plain drinking water), GTP group (GTP, 100 mg kg^{-1} body weight), PHGG group (PHGG, 100 mg kg^{-1} body weight), GTP plus PHGG group (GTP, 50 mg kg^{-1} body weight plus PHGG, 50 mg kg^{-1} body weight). The administration dose was determined on the basis of other reports (Yamamoto et al 2000; Sabu et al 2002). A normal group of rats that underwent a sham operation and did not receive STZ was also included. Each experimental group contained 10 rats. After 50 days of treatment, urine was collected and blood samples were obtained by cardiac puncture. The serum was immediately separated from the blood samples by centrifugation. After renal perfusion through the renal artery with ice-cold physiological saline, the kidneys were removed from the rats and one part of the tissues was immersed in formalin for histological examination and the other part was kept at −80°C until analysis.

Assays of serum and urine samples

Serum glucose, urea nitrogen, creatinine (Cr), albumin, triglyceride and total cholesterol were determined using commercial reagents (Glucose CII-Test Wako, A/G B-Test Wako, Triglyceride E-Test Wako and Cholesterol E-Test Wako obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan; BUN Kainos and CRE-EN Kainos obtained from Kainos Laboratories Inc., Tokyo, Japan). Serum glycosylated protein and malondialdehyde (MDA) levels were measured using the methods of McFarland et al (1979) and Naito & Yamanaka (1978), respectively. Urine component levels were determined as follows: Cr using a commercial reagent (CRE-EN Kainos obtained from Kainos Laboratories, Inc.) and protein by the sulfosalicylic acid method (Sakagishi 1968). The creatinine clearance (Ccr) was calculated on the basis of urinary Cr, serum Cr, urine volume and body weight using the following equation: Ccr (mL min^{-1} kg^{-1} body weight) = (urinary Cr (mg dL^{-1}) × urine volume (mL)/serum Cr (mg dL^{-1})) × (1000/body weight (g)) × (1/1440 (min)).

Determination of MDA level

For assay of MDA, the kidney tissue was homogenized with a nine-fold volume of ice-cold 1.15% KCl. The MDA level of each homogenate was measured according to the method of Mihara & Uchiyama (1978), based on the reaction with thiobarbituric acid.

Superoxide dismutase (SOD) activity assay

The kidney tissue was homogenized with a nine-fold volume of ice-cold physiological saline and the activity of the enzymes in the homogenate was determined. The activity of SOD was measured according to the nitrous acid method described by Elstner & Heupel (1976) and Oyanagui (1984), which is based on inhibition of nitrite formation from hydroxylamine in the presence of superoxide (O_2^-) generators.

Determination of protein level

Protein levels were evaluated by the micro-biuret method (Itzhaki & Gill 1964) with bovine serum albumin as the standard.

Histological examination

Renal tissues were fixed in 10% neutral-buffered formalin solution and embedded in paraffin. The tissues were then cut into 4-μm sections, mounted on silane-coated glass slides, and stained with haematoxylin–eosin, periodic acid–Schiff reagent, periodic acid–methenamine silver and phosphotungstic acid–haematoxylin. Two hundred or fewer glomeruli in each sample were examined by light microscopy, and exudative and diffuse lesions were scored histologically as 0 = 0%, 1 = 0–5%, 2 = 5–10%, or 3 = >10% of the total glomeruli.

Statistics

The results for each group are expressed as mean ± s.e. The effect on each parameter was examined using the one-way analysis of variance. Differences among groups were evaluated by Dunnett's test and those at $P < 0.05$ were considered to be statistically significant.

Results

Body and kidney weights

As shown in Table 1, the body weight of the control rats with diabetic nephropathy was significantly lower than that of the normal rats, and there were no significant differences between the untreated control group and the three treated groups. Compared with untreated control rats, kidney weight was significantly reduced in the rats treated with GTP plus PHGG.

General biochemical parameters

Table 2 shows the effect of each treatment on levels of serum glucose and glycosylated protein, which were both markedly elevated in rats with diabetic nephropathy. The GTP and PHGG groups showed significantly reduced blood glucose levels at 50 days, but did not show any change in their levels of glycosylated protein. On the other hand, the group treated with GTP plus PHGG showed significant decreases in both blood glucose and glycosylated protein levels, as shown in Table 2.

The serum Cr level was significantly increased in rats with diabetic nephropathy in comparison with normal rats, as shown in Table 3. Among the three treated groups, those given GTP plus PHGG showed the greatest reduction level at 25 days, and this effect was evident even after 50 days. The GTP group also showed a significant reduction in Cr, whereas the PHGG group showed no change. On the other hand, rats with diabetic nephropathy showed a significant reduction in Ccr in comparison with normal rats. The GTP plus PHGG group showed a significant increase in this value at 50 days, as shown in Table 3.

At 25 days, urinary protein excretion in untreated control rats with diabetic nephropathy was about 28.2 mg day^{-1}, as shown in Figure 1, and this further increased to 40.8 mg day^{-1} at 50 days. The increase in urinary protein levels was significantly reduced in all three treatment groups.

Table 4 shows the effects of treatment on serum parameters under conditions of diabetic nephropathy. The urea nitrogen level increased from 18.0 to 46.1 mg dL^{-1} in controls, whereas it was reduced significantly to 38.5 mg dL^{-1} by administration of GTP plus PHGG. The decreased level of albumin under conditions of diabetic nephropathy was normalized by administration of GTP plus PHGG. The control rats with diabetic nephropathy had much higher levels of triglyceride and total cholesterol than normal rats, while each of the treatments significantly reduced the triglyceride level. Additionally, administration of PHGG significantly reduced the level of serum cholesterol.

MDA levels in serum and kidney

As shown in Tables 4 and 5, MDA levels in serum and kidney were higher in the untreated control rats than in normal rats. After 50 days, all three treatments significantly decreased the serum MDA level, whereas the renal MDA level tended to be decreased by GTP treatment.

Table 1 Body and kidney weights

Group	Body weight (BW)			Kidney weight (g 100 g^{-1} BW)
	Initial (g)	Final (g)	Gain (g)	
Normal rats	306.0 ± 5.4	374.8 ± 18.1	68.8 ± 13.1	0.590 ± 0.029
Diabetic nephropathy rats				
Control	248.5 ± 4.7$^{\#}$	275.1 ± 8.7$^{\#}$	23.7 ± 6.2$^{\#}$	0.643 ± 0.027
GTP (100 mg kg^{-1} BW day^{-1})	250.4 ± 7.0$^{\#}$	270.9 ± 16.0$^{\#}$	34.0 ± 7.1$^{\#}$	0.627 ± 0.037
PHGG (100 mg kg^{-1} BW day^{-1})	246.0 ± 5.9$^{\#}$	260.9 ± 15.3$^{\#}$	29.9 ± 11.4$^{\#}$	0.605 ± 0.028
GTP plus PHGG (50 mg kg^{-1} BW day^{-1} plus 50 mg kg^{-1} BW day^{-1})	248.3 ± 5.9$^{\#}$	267.4 ± 9.7$^{\#}$	25.5 ± 4.0$^{\#}$	0.581 ± 0.016*

$^{\#}P < 0.001$ vs normal rats; *$P < 0.01$ vs control rats with diabetic nephropathy.

Table 2 Serum glycaemic condition

Group	Glucose ($mg\,dL^{-1}$)		Glycosylated protein ($nmol\,mg^{-1}$ protein)
	25 days	50 days	50 days
Normal rats	140.9 ± 4.2	169.1 ± 6.6	18.9 ± 0.3
Diabetic nephropathy rats			
Control	$606.0 \pm 52.9^{\#}$	$667.0 \pm 39.5^{\#}$	$29.8 \pm 0.9^{\#}$
GTP ($100\,mg\,kg^{-1}$ BW day^{-1})	$618.2 \pm 46.3^{\#}$	$574.9 \pm 53.0^{\#}$**	$28.6 \pm 2.2^{\#}$
PHGG ($100\,mg\,kg^{-1}$ BW day^{-1})	$579.4 \pm 49.8^{\#}$	$580.5 \pm 43.5^{\#}$*	$29.3 \pm 1.8^{\#}$
GTP plus PHGG ($50\,mg\,kg^{-1}$ BW day^{-1} plus $50\,mg\,kg^{-1}$ BW day^{-1})	$551.7 \pm 2.9^{\#}$	$538.3 \pm 21.8^{\#}$***	$26.1 \pm 0.7^{\#}$**

$^{\#}P < 0.001$ vs normal rats; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs control rats with diabetic nephropathy.

Table 3 Serum Cr and Ccr

Group	Cr ($mg\,dL^{-1}$)		Ccr ($mL\,kg^{-1}$ BW min^{-1})	
	25 days	50 days	25 days	50 days
Normal rats	0.254 ± 0.017	0.384 ± 0.005	9.62 ± 0.63	6.46 ± 0.37
Diabetic nephropathy rats				
Control	$0.506 \pm 0.005^{\#}$	$0.534 \pm 0.026^{\#}$	$4.27 \pm 0.42^{\#}$	$3.59 \pm 0.26^{\#}$
GTP ($100\,mg\,kg^{-1}$ BW day^{-1})	$0.471 \pm 0.026^{\#}$*	$0.496 \pm 0.014^{\#}$**	$4.25 \pm 0.40^{\#}$	$3.64 \pm 0.18^{\#}$
PHGG ($100\,mg\,kg^{-1}$ BW day^{-1})	$0.482 \pm 0.026^{\#}$	$0.537 \pm 0.027^{\#}$	$4.00 \pm 0.19^{\#}$	$3.55 \pm 0.22^{\#}$
GTP plus PHGG ($50\,mg\,kg^{-1}$ BW day^{-1} plus $50\,mg\,kg^{-1}$ BW day^{-1})	$0.433 \pm 0.003^{\#}$***	$0.466 \pm 0.012^{\#}$***	$4.22 \pm 0.02^{\#}$	$4.10 \pm 0.17^{\#}$**

$^{\#}P < 0.001$ vs normal rats; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs control rats with diabetic nephropathy.

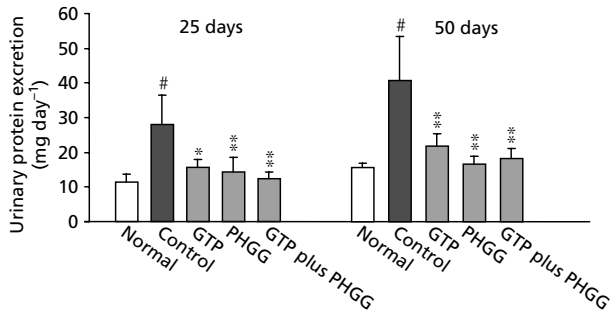

Figure 1 Urinary protein excretion. $^{\#}P < 0.001$ vs normal rats; $*P < 0.01$, $**P < 0.001$ vs control rats with diabetic nephropathy.

Renal SOD activity

As shown in Table 5, the renal SOD activity in the untreated control rats was significantly lower than that in normal rats. After administration of GTP, the renal SOD activity was normalized.

Histological findings

Table 6 shows the results of histopathological examination of the kidneys. The severity of renal damage was evaluated by assigning lesion scores, as described in the Methods section. The kidneys of rats with diabetic nephropathy developed diffuse and exudative lesions, while advanced diabetic glomerulosclerosis with fibrin caps and capsular drops was less observed. As shown in Table 6, the diffuse lesion score in untreated control rats was 1.60. After treatment for 50 days, this score was reduced to 0.75, 1.10 and 0.89, respectively, in the rats given GTP, PHGG and GTP plus PHGG. In addition, the scores for exudative lesions were also reduced to 0.75 and 0.70 in the groups given GTP and PHGG, respectively, compared with 1.20 in the untreated control rats. Administration of GTP plus PHGG further lowered the score to 0.56.

Representative photomicrographs of the glomeruli obtained from each group are shown in Figure 2. The normal rats that underwent a sham operation had normal renal glomerular morphology (Figure 2A). In contrast, the glomeruli of rats with diabetic nephropathy were enlarged due to diabetic exudative and diffuse lesions. All the treated groups showed a reduced severity of morphological changes and lesion score compared with the untreated control rats (Figure 2B–E).

Discussion

Recently, the advantageous use of functional components of foods for people with lifestyle-related diseases, including

Table 4 Serum biochemical features

Group	Urea nitrogen ($mg\,dL^{-1}$)	Albumin ($g\,dL^{-1}$)	Triglyceride ($mg\,dL^{-1}$)	Total cholesterol ($mg\,dL^{-1}$)	MDA ($nmol\,mL^{-1}$)
Normal rats	18.0 ± 1.6	3.47 ± 0.04	193.0 ± 2.1	69.6 ± 5.5	1.47 ± 0.20
Diabetic nephropathy rats					
Control	$46.1 \pm 3.7^{\#\#}$	$2.97 \pm 0.07^{\#\#}$	$256.3 \pm 27.3^{\#}$	$138.0 \pm 17.6^{\#\#}$	$2.72 \pm 0.17^{\#\#}$
GTP ($100\,mg\,kg^{-1}$ BW day^{-1})	$43.9 \pm 2.9^{\#\#}$	$3.01 \pm 0.10^{\#\#}$	201.8 ± 12.4*	$127.5 \pm 10.1^{\#\#}$	$2.13 \pm 0.11^{\#\#}$**
PHGG ($100\,mg\,kg^{-1}$ BW day^{-1})	$41.4 \pm 3.8^{\#\#}$	$3.08 \pm 0.06^{\#\#}$	202.4 ± 33.9*	$112.0 \pm 8.9^{\#\#}$*	$2.31 \pm 0.13^{\#\#}$**
GTP plus PHGG ($50\,mg\,kg^{-1}$ BW day^{-1} plus $50\,mg\,kg^{-1}$ BW day^{-1})	$38.5 \pm 2.3^{\#\#}$*	$3.22 \pm 0.05^{\#\#}$**	201.7 ± 19.6*	$125.8 \pm 5.9^{\#\#}$	$2.18 \pm 0.13^{\#\#}$**

$^{\#}P < 0.01$, $^{\#\#}P < 0.001$ vs normal rats; $^{*}P < 0.01$, $^{**}P < 0.001$ vs control rats with diabetic nephropathy.

Table 5 MDA levels and SOD activity in kidney

Group	MDA ($nmol\,mg^{-1}$ protein)	SOD activity ($U\,mg^{-1}$ protein)
Normal rats	0.453 ± 0.033	35.0 ± 1.6
Diabetic nephropathy rats		
Control	$0.654 \pm 0.019^{\#\#\#}$	$31.9 \pm 1.4^{\#}$
GTP ($100\,mg\,kg^{-1}$ BW day^{-1})	$0.605 \pm 0.026^{\#\#\#}$	36.2 ± 2.2*
PHGG ($100\,mg\,kg^{-1}$ BW day^{-1})	$0.647 \pm 0.056^{\#\#\#}$	$30.0 \pm 1.9^{\#\#}$
GTP plus PHGG ($50\,mg\,kg^{-1}$ BW day^{-1} plus $50\,mg\,kg^{-1}$ BW day^{-1})	$0.642 \pm 0.011^{\#\#\#}$	$29.4 \pm 1.2^{\#\#\#}$

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs normal rats; $^{*}P < 0.01$ vs control rats with diabetic nephropathy.

Table 6 Histological evaluation of the kidney

Group	Lesion score	
	Diffuse lesion	Exudative lesion
Normal rats	0.00 ± 0.00	0.00 ± 0.00
Diabetic nephropathy rats		
Control	1.60 ± 0.16	1.20 ± 0.36
GTP ($100\,mg\,kg^{-1}$ BW day^{-1})	0.75 ± 0.25***	0.75 ± 0.31
PHGG ($100\,mg\,kg^{-1}$ BW day^{-1})	1.10 ± 0.23**	0.70 ± 0.21*
GTP plus PHGG ($50\,mg\,kg^{-1}$ BW day^{-1} plus $50\,mg\,kg^{-1}$ BW day^{-1})	0.89 ± 0.11***	0.56 ± 0.24**

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control rats with diabetic nephropathy.

diabetes and its complications, has been receiving much attention. In this study, to investigate the possible benefits of GTP and dietary fibre for diabetic nephropathy, we employed model rats that had been subjected to subtotal nephrectomy followed by STZ injection. This rat model shows metabolic abnormalities and renal lesions resembling those seen in patients with diabetic nephropathy (Yokozawa et al 2001).

Hyperglycaemia is the primary clinical manifestation of diabetes. Strict control of the plasma glucose concentration by insulin treatment has been shown to prevent renal hypertrophy and the subsequent increase in urinary protein excretion (Rasch 1980), supporting the importance of controlling the blood glucose level for primary prevention of diabetic nephropathy and possibly for slowing or even reversing some of the early abnormalities. Consumption of guar gum is known to improve the postprandial rise of blood glucose and insulin levels in the early stage of the disease (Jenkins et al 1977; Daumerie & Henquin 1982). Additionally, Gallaher & Schaubert (1990) have demonstrated improved long-term blood glucose control in diabetic rats fed guar gum for 4 weeks, as indicated by a lower percentage of glycated haemoglobin. In this study, we used rats with diabetic nephropathy in which renal dysfunction was already evident. PHGG treatment for 50 days lowered their blood glucose levels, but did not change the levels of glycosylated protein, which reflect the time-averaged blood glucose concentration over the preceding 2 weeks. The GTP-treated group showed similar changes to those in the PHGG-treated group. On the other hand, a combination of GTP and PHGG reduced the serum glucose level and the extent of glycosylation of serum protein, suggesting an ability to slow the progression of diabetic nephropathy.

Proteinuria is a powerful predictor of nephropathy in patients with diabetes. The increased urinary protein excretion results from a lesion in the glomerular basement membrane. In comparison with normal rats, rats with diabetic nephropathy showed increased urinary excretion of protein as the experimental period was prolonged, indicating the progression of renal dysfunction. Administration of GTP, PHGG and GTP plus PHGG for 50 days significantly reduced the degree of proteinuria. The groups treated with GTP and GTP plus PHGG showed reduced serum Cr levels, and the latter treatment resulted in the lowest value of serum Cr, and also improved the serum urea nitrogen level. The Ccr level in rats with diabetic nephropathy was about half of that in normal rats. In patients with diabetes and/or renal failure, Ccr is an effective index of the glomerular filtration rate. However, rats with diabetic nephropathy given GTP plus PHGG showed a recovery towards normal values to some

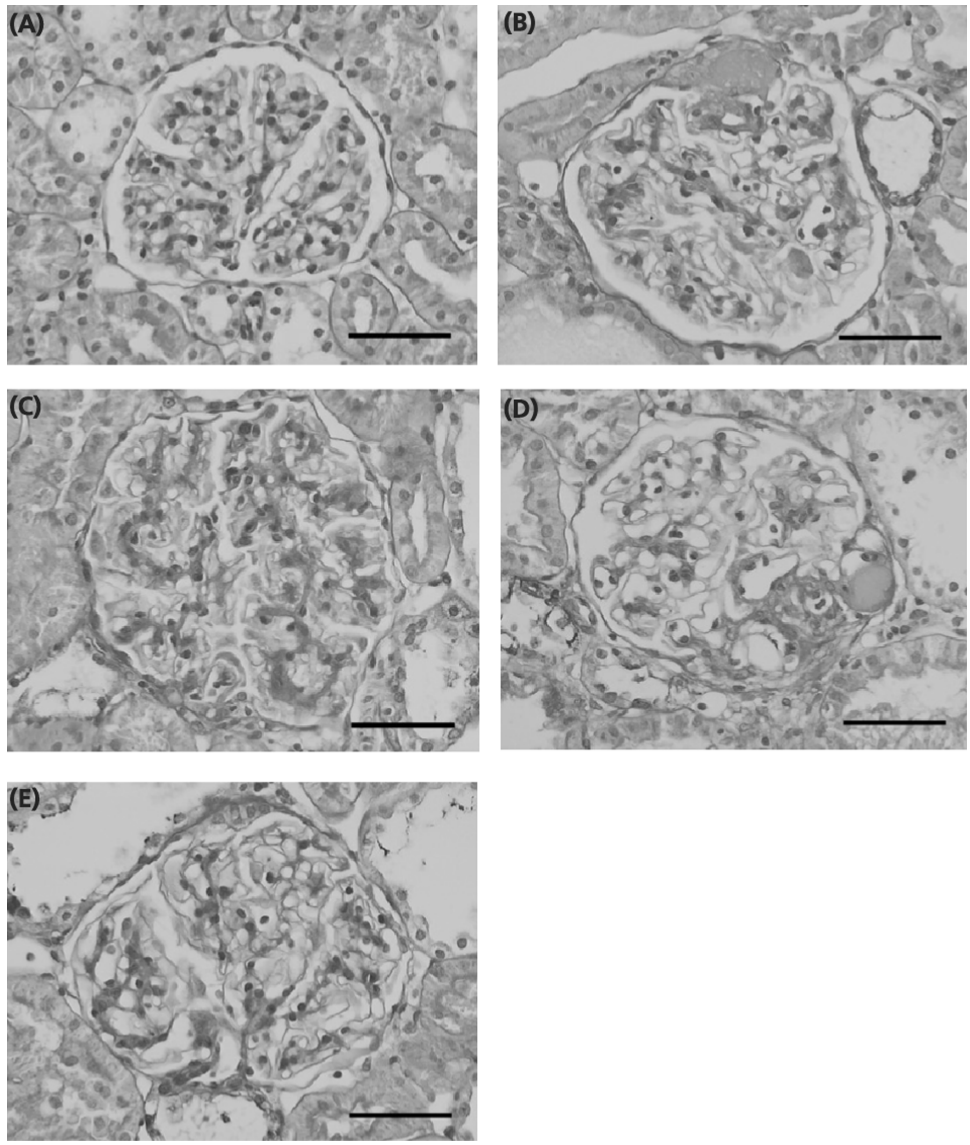

Figure 2 Photomicrographs of the glomeruli obtained from normal rats (A), diabetic nephropathy rats in the control (B), GTP (C), PHGG (D), and GTP plus PHGG (E) treated groups. Scale bar = 50 μm.

extent, while the GTP and PHGG groups showed no change in Ccr. It is well known that the glomerular filtration rate is lowest in the diabetic patients with the lowest serum albumin (Nawata et al 2004). Accordingly, the improvement of Ccr by GTP plus PHGG treatment group may bring about the increased serum albumin level in this group. Kidney weight was also reduced by administration of GTP plus PHGG. Diabetic nephropathy is characterized by excessive deposition of extracellular matrix proteins in the glomeruli and subsequent mesangial expansion, resulting in renal dysfunction. All three treated groups had fewer of the diffuse and exudative lesions in the glomeruli associated with mesangial expansion. The above results reflecting renal function and morphology indicate that GTP, PHGG and GTP plus PHGG all delay the progression of diabetic nephropathy. In particular, the combination of GTP and PHGG may exert relatively strong renoprotective activity.

Recent studies have extensively discussed the likelihood that persistent hyperglycemia is responsible for oxidative stress and is associated with the development of diabetic nephropathy (Baynes & Thorpe 1999; Ha & Kim 1999). Glucose itself, and glycosylated proteins, are susceptible to autoxidation and may be a source of reactive oxygen species (ROS) (Mullarkey et al 1990). Furthermore, enhanced ROS due to diabetes may result from a dysfunction in the defence system against oxidative stress, such as reduction of glutathione (Tachi et al 2001) or inactivation of free-radical-scavenging enzymes (Wohaieb & Godin 1987). Excessive production of ROS is widely recognized to have a harmful influence on the body because ROS injure lipids, proteins and nucleic acids, leading to structural and

functional impairments. Under the conditions of diabetes, increased levels of oxidation products such as malondialdehyde, advanced glycation of proteins and 8-hydroxy-2′-deoxyguanosine have been observed in many experimental and clinical studies (Vlassara et al 1994; Ha & Kim 1995; Kakimoto et al 2002), indicating that diabetes can be regarded as a disease related to increased oxidative stress. It has therefore been suggested that a variety of antioxidants that eliminate oxidative stress may attenuate the pathological conditions induced by hyperglycaemia and oxidative stress, thereby preventing the development of diabetic complications.

Green tea has become a very popular antioxidant neutraceutical, following the recent recognition that oxidative stress is closely associated with the pathogenesis of many clinical disorders. GTP are known to scavenge free radicals and inhibit lipid peroxide formation (Salah et al 1995; Guo et al 1996). Previously, using nephrectomized rats, we demonstrated that GTP decrease lipid peroxide and ameliorate alterations to free-radical-scavenging enzymes in the kidney (Yokozawa et al 1996). Wohaieb & Godin (1987) reported that altered activities of free-radical-scavenging enzymes are also linked to enhanced oxidative stress under diabetic conditions. SOD, which is a scavenger of O_2^-, plays an important role in the endogenous defence system against oxygen free radicals. A number of experimental and clinical studies have suggested that SOD activity in the kidney of diabetic rats is decreased (Godin et al 1988). In the present study, therefore, we measured lipid peroxidation levels and SOD activity in the kidney to examine the effects of GTP on oxidative stress. We found that GTP exerted antioxidative activity in rats with diabetic nephropathy, reducing serum lipid peroxidation and increasing SOD activity. Renal lipid peroxidation levels showed a non-significant tendency to be reduced by GTP treatment. The other two treatments, PHGG and GTP plus PHGG, also reduced serum lipid peroxidation levels, although there were no changes in kidney lipid peroxidation levels or SOD activity.

The importance of dietary fibre consumption has been highlighted by epidemiological and clinical studies in the field of preventive medicine (Spiller 1994). In addition to the beneficial effects on glycaemic control, it has been well demonstrated that dietary fibre lowers the serum concentrations of cholesterol and triglyceride (Chen & Anderson 1979). Hyperlipidaemia is observed in patients with diabetic nephropathy (Mulec et al 1993; Kramer-Guth et al 1996) and is also a causative factor of glomerular sclerosis. Our results that serum cholesterol and triglyceride levels were significantly reduced by PHGG administration agreed with above reports, which suggested that PHGG lessens the development of diabetic nephropathy because of its hypolipidaemic effect. Although GTP are also reported to have a cholesterol-lowering effect in cholesterol-fed rats (Yokozawa et al 2002), GTP administration along with GTP plus PHGG administration did not change it in the present study. It may be that the administered doses of GTP (100 mg kg^{-1} body weight day^{-1}) and GTP plus PHGG (50 mg kg^{-1} body weight day^{-1} plus 50 mg kg^{-1} body weight day^{-1}) are too low to exert this effect.

Taking the present results as a whole, it is speculated that GTP and PHGG exert antioxidative and hypolipidaemic activity, respectively, thus helping to reduce the risk of diabetic nephropathy and slowing the decline of renal function. Thus, a combination of GTP and PHGG can bring about a wide range of improvements in the pathological conditions related to diabetic nephropathy, and can exert renoprotective activity. Further experiments to determine the mechanisms by which GTP and PHGG slow the progression of diabetic nephropathy will be necessary to clarify in more detail the properties of these functional components of foods. Our present findings support the idea that dietary GTP and PHGG may be beneficial for the management of patients with diabetic nephropathy.

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