Novel approaches to oxidative stress-induced renal failure: Therapeutic potentials of Sanguisorbae Radix, Wen-Pi-Tang and green tea

Takako YOKOZAWA,* Eun Ju CHO, Dong Young RHYU and Takako NAKAGAWA

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan.

(Accepted February 24, 2003.)

Abstract

Oxidative stress has been suggested to be one of the major causes of degenerative diseases, including renal failure. Therefore, antioxidant therapy to prevent the progression of renal failure and its related complications has attracted much attention. Although there are several synthetic antioxidants, in recent years, great effort has been focused on the use of natural phytochemicals present in herbs and foods because of the toxicity and side effects of synthetic antioxidants. This review summarizes the potential protective activities of two Chinese traditional medicines, Sanguisorbae Radix and Wen-Pi-Tang, and green tea, which are rich sources of polyphenols, against renal failure. Sanguisorbae Radix and its main active component sanguiin H-6 showed protective activity against NO-induced renal failure without toxicity. In addition, the Chinese prescription Wen-Pi-Tang and ECg regulated $ONOO^-$ formation and exerted beneficial effects against $ONOO^-$-induced oxidative injury and renal dysfunction. Green tea polyphenols also showed antioxidative activities in *in vitro* and *in vivo* experimental systems, and a clinical study suggested that they are useful for the treatment of renal injury. Even though the synthetic antioxidants are not always effective in improving renal failure due to their toxicity and/or side effects together with beneficial effects, Sanguisorbae Radix, Wen-Pi-Tang and green tea polyphenols displayed antioxidative activities against oxidative stress-induced renal failure without side effects. We expect the information presented in this review to help provide novel approaches, with low toxicity, to the prevention and effective treatment of renal diseases.

Key words renal failure, oxidative stress, Sanguisorbae Radix, Wen-Pi-Tang, green tea.

1. Introduction

Renal disease is one of the major health problems associated with considerable increases in morbidity and mortality with a reduced quality of life. Therapy with angiotensin converting enzyme inhibitors, protein restriction, dialysis and renal transplantation are the commonly employed management strategies for renal diseases.[1] However, the number of patients with renal failure, especially patients undergoing dialysis therapy and with end-stage renal disease, is growing worldwide, which implies that we have no effective strategy to halt the progression of renal diseases. In addition, the dialysis procedure carries the risk of bleeding and hemorrhage from the site of vascular access.[2] Furthermore, given the costs of dialysis and transplantation together with the high morbidity and mortality from end-stage renal failure, there is a need for therapeutic advances and new approaches to prevent and treat effectively renal diseases and their associated complications. Thus, the focus in recent years has shifted to optimizing the care of these patients during the phase of chronic renal disease, before the onset of end-stage renal disease. It is critical for patients with chronic renal disease to slow the rate of progression of renal failure and prevent its related complications. In this review, we provide novel suggestions for the management of renal diseases.

Although several causes of renal failure have been demonstrated, in recent years, numerous clinical and

*To whom correspondence should be addressed. e-mail : yokozawa@ms.toyama-mpu.ac.jp

experimental studies have indicated that increased oxidative stress is mainly responsible for renal failure.[3,4] In addition, several studies demonstrated that active oxygen species resulted in histological lesions such as glomerular sclerosis, tubulointerstitial changes and mesangial matrix expansion under renal failure.[5,6] Since the involvement of oxidative stress at the onset of renal failure has been well established, the associated pathological conditions could be improved by the amelioration of oxidative stress through treatment with scavengers of the hydroxyl radical (·OH), superoxide anion (O_2^-), nitric oxide (NO) and peroxynitrite ($ONOO^-$) and enhancement of the antioxidative defense system. Therefore, it is considered important to search for oxygen radical scavengers that can play crucial roles in effective defense against free radical-related diseases, including renal failure. In this review, we focus on oxidative stress-induced renal failure and, based on the findings of our previous studies, discuss the protective roles of Chinese traditional medicines and green tea.

Traditional crude drugs that are usually derived from natural sources have been employed for thousands of years in Chinese medicine and their prescriptions have played significant roles in the promotion of human health and treatment of various diseases. The World Health Organization estimated that about 80% of Earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of the crude drugs or their active components.[7] Traditional medicines are also considered to be potential sources of new therapeutic agents and medicines because of their distinctive biological activities associated with low toxicity. Furthermore, numerous crude drugs or their constituents, both *in vitro* and *in vivo*, markedly suppressed lipid peroxidation and scavenged reactive free radicals, which are thought to contribute to their therapeutic effects against oxidative stress.[8-13] Our clinical study showed that, in patients with chronic renal failure, traditional Chinese prescriptions, in particular Wen-Pi-Tang, effectively reduced serum creatinine (Cr) levels and retarded the progression of chronic renal failure.[14] These findings suggest that research into traditional Chinese prescriptions and their crude drugs would have great potential for the management of renal failure. The discriminate and proper use of some traditional Chinese prescriptions and their active components is expected to be safe and have therapeutic benefits.

Besides traditional Chinese prescriptions and crude drugs, dietary sources with antioxidant activities have also received particular attention because of their potential roles in modulating oxidative stress-induced pathological conditions. Several studies have supported the roles of dietary antioxidants in protecting against free radicals, eventually resulting in disease prevention or overall health promotion.[15-18] In particular, various teas contain several kinds of biologically active phytochemicals, such as polyphenols, that can provide therapeutic effects. They have extensive biological properties of value to the promotion of human health and reduction of the risk of disease. Epidemiological studies have shown that polyphenols present in green tea contribute to reducing the risk of oxidative stress-related diseases.[19-21]

In this review, we describe the potential therapeutic activities of Sanguisorbae Radix, a traditional crude drug, Wen-Pi-Tang, a Chinese prescription, and green tea, which contains high levels of polyphenols, against oxidative stress-induced renal damage.

2. Activities of Sanguisorbae Radix against NO-induced renal injury

Sanguisorbae Radix is used for several disorders, such as hemostasis, hematemesis, hemoptysis, melena, hypermenorrhea, dermatitis and eczema, even though there is no scientific evidence to support its use. Our serial studies on Sanguisorbae Radix both *in vitro* and *in vivo* showed that it possesses strong free radical-scavenging activity.[22-29] In particular, the extract showed protective activity against oxidative stress-related renal diseases and antioxidative potential in senescence-accelerated mice. These findings suggest that Sanguisorbae Radix would be an effective agent for the amelioration of pathological conditions related to excessive generation of free radicals and oxidative damage. In this section, we discuss the protective activities of Sanguisorbae Radix and its main active component against NO-induced renal damage induced by lipopolysaccharide (LPS).

2.1. Effects on renal dysfunction induced by LPS: NO is a biologically important molecule which acts in various physiological and pathological process in differ-

ent organs. In the kidney, NO plays an important role in the regulation of renal hemodynamics, sodium excretion, renin release, tubuloglomerular feedback, pressure natriuresis and tubular function. Both over- and under-production of NO have been implicated in various renal pathologies, including acute and chronic renal failure, various types of nephritis and diabetic nephropathy.[30-33] The pathophysiological importance of NO suggests that regulation of NO formation may be an efficient strategy for intervention to improve or alleviate these pathological conditions. Therefore, we tried to search for modulators of NO formation among traditional crude drugs by systematically screening for direct NO-scavenging activity. We found that Sanguisorbae Radix, a traditional crude drug which contains a large amount of polyphenols, its major constituents, was the most effective scavenger of NO. On the basis of the *in vitro* results, we investigated whether Sanguisorbae Radix protected against NO-induced renal failure stimulated by LPS in an *in vivo* system.

Rats treated with LPS, which results in excessive NO production, showed renal dysfunction, which was assessed by increases in renal functional parameters, i.e., urea nitrogen and Cr levels in serum (Table I). In addition, the serum nitrite/nitrate level, an indicator of NO formation, was also markedly increased in LPS-treated rats compared with that seen in normal rats (Table II). The excessive production of NO caused by LPS is considered to contribute to impairment of renal function. However, the administration of Sanguisorbae Radix extract led to a decrease in NO production and thus ameliorated renal impairment through the reductions in serum urea nitrogen and Cr levels.

NO is produced from L-arginine by the action of NO synthase (NOS). In the kidney, three isoforms of NOS, which exhibit distinct functions in different regions of the kidney, have been found.[34] Excessive NO is produced mainly by inducible NOS (iNOS). Therefore, blocking the cytotoxicity of NO may be achieved by suppressing iNOS activity and/or scavenging NO. Under normal conditions, iNOS also generates physiological amounts of NO, which may participate in the modulation of vascular tone by an indirect mechanism involved in mesangial cell relaxation. However, in the presence of certain cytokines and under conditions of hypoxia, NO is generated in large quantities for a prolonged period.[35] In addition, since excessive generation of NO in renal disease is mainly associated with the induction of iNOS,

Table I Effect of Sanguisorbae Radix extract on urea nitrogen and Cr levels in serum.

Group	Urea nitrogen (mg/dl)	Cr (mg/dl)
Normal	21.3 ± 2.0	0.36 ± 0.01
LPS-treated		
Control	38.1 ± 2.9[a]	1.20 ± 0.08[a]
Sanguisorbae Radix extract (50 mg/kg B.W./day)	33.8 ± 3.1[a,b]	0.78 ± 0.10[a,d]
Sanguisorbae Radix extract (100 mg/kg B.W./day)	31.6 ± 2.3[a,d]	0.68 ± 0.08[a,d]
LPS-treated		
Control	37.8 ± 1.6[a]	1.18 ± 0.06[a]
Aminoguanidine (5 mg/kg plus 5 mg/kg B.W./h)	32.4 ± 2.8[a,c]	0.66 ± 0.14[a,d]

[a]$p<0.001$ *vs.* normal values, [b]$p<0.05$, [c]$p<0.01$, [d]$p<0.001$ *vs.* LPS-treated control values.

Table II Effect of Sanguisorbae Radix extract on nitrite/nitrate level in serum.

Group	Nitrite/nitrate (μM)
Normal	1.78 ± 1.02
LPS-treated	
Control	6.50 ± 1.35[b]
Sanguisorbae Radix extract (50 mg/kg B.W./day)	4.39 ± 1.82[b,c]
Sanguisorbae Radix extract (100 mg/kg B.W./day)	3.72 ± 0.89[a,c]
LPS-treated	
Control	6.39 ± 1.24[b]
Aminoguanidine (5 mg/kg plus 5 mg/kg B.W./h)	3.13 ± 1.28[c]

[a]$p<0.01$, [b]$p<0.001$ *vs.* normal values, [c]$p<0.001$ *vs.* LPS-treated control values.

Table III Effect of Sanguisorbae Radix extract on iNOS activity in kidney.

Group	iNOS (pmol/mg protein/min)
Normal	1.94 ± 0.11
LPS-treated	
Control	3.67 ± 0.27[b]
Sanguisorbae Radix extract (50 mg/kg B.W./day)	2.69 ± 0.10[a,c]
Sanguisorbae Radix extract (100 mg/kg B.W./day)	2.58 ± 0.06[a,c]
LPS-treated	
Control	3.64 ± 0.29[b]
Aminoguanidine (5 mg/kg plus 5 mg/kg B.W./h)	2.02 ± 0.18[c]

[a]$p<0.01$, [b]$p<0.001$ *vs.* normal values, [c]$p<0.001$ *vs.* LPS-treated control values.

Table IV NO production, iNOS activity, NADPH-diaphorase activity and cell viability of macrophages incubated with LPS.

Group	NO (μM)	iNOS (pmol/mg protein/min)	NADPH-diaphorase (nmol/mg protein)	Cell viability (%)
None	4.43 ± 0.13	6.83 ± 1.59	23.72 ± 0.75	100.0 ± 2.1
LPS-treatment				
Control	51.50 ± 0.30^{c}	26.42 ± 1.64^{c}	47.05 ± 4.60^{c}	69.8 ± 4.6^{c}
Extract (25 μg/ml)	42.00 ± 0.46^{c,e}	22.88 ± 1.52^{c,d}	44.93 ± 3.88^{c}	72.8 ± 2.1^{c}
Extract (50 μg/ml)	32.51 ± 0.25^{c,e}	20.54 ± 1.09^{c,e}	35.17 ± 2.04^{c,e}	74.4 ± 1.3^{c}
Extract (100 μg/ml)	21.55 ± 0.22^{c,e}	16.81 ± 1.93^{c,e}	30.45 ± 2.52^{a,e}	77.9 ± 0.5^{c,e}
LPS-treatment				
Control	47.80 ± 0.33^{c}	26.08 ± 1.52^{c}	50.01 ± 3.09^{c}	69.5 ± 3.2^{c}
Sanguiin H-6 (50 μg/ml)	9.03 ± 0.27^{c,e}	8.74 ± 1.59^{e}	11.44 ± 1.56^{c,e}	92.4 ± 0.9^{b,e}
Sanguiin H-11 (50 μg/ml)	11.27 ± 0.89^{c,e}	10.41 ± 1.67^{b,e}	16.71 ± 1.64^{c,e}	89.5 ± 3.5^{c,e}
1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose (50 μg/ml)	10.74 ± 0.45^{c,e}	9.32 ± 0.42^{e}	13.02 ± 1.45^{c,e}	82.8 ± 0.9^{c,e}
Eugeniin (50 μg/ml)	12.87 ± 0.55^{c,e}	15.73 ± 1.26^{c,e}	25.10 ± 2.40^{e}	81.1 ± 2.4^{c,e}
Polymeric proanthocyanidin (50 μg/ml)	14.85 ± 0.85^{c,e}	19.29 ± 1.67^{c,e}	29.70 ± 1.10^{c,e}	83.7 ± 4.2^{c,e}
Aminoguanidine (100 μM)	8.99 ± 0.10^{c,e}	8.98 ± 0.53^{e}	10.91 ± 0.89^{c,e}	73.7 ± 1.3^{c}

$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ *vs.* none treatment values, $^{d}p<0.05$, $^{e}p<0.001$ *vs.* LPS-treatment control values.

therapeutic strategies have concentrated on the development of effective iNOS inhibitors. As shown in Table III, LPS treatment resulted in an approximately 1.9-fold increase in iNOS activity, suggesting the possible association of additional induction of iNOS with NO generation and renal dysfunction. However, Sanguisorbae Radix extract resulted in decreased renal iNOS activity, although its effect was weaker than that of aminoguanidine, a selective iNOS inhibitor.

2.2. Active components with NO production-suppressing activity : The active components of an aqueous extract of Sanguisorbae Radix were determined in experiments using macrophages that were activated by the addition of LPS. The macrophages of mice given LPS showed greatly increased amounts of NO together with increases in the activities of iNOS and NADPH-diaphorase, which is used as a histochemical marker of neuronal NOS,[36] whereas the cell viability decreased. Mitchell *et al.*[37] have also published data showing that in macrophages, both NADPH-diaphorase and NOS activities can be induced by LPS. In our study, we confirmed that NADPH-diaphorase and iNOS activities were increased by LPS treatment, consistent with the findings of Tracey *et al.*[38] In contrast, in macrophages treated with stepwise doses of Sanguisorbae Radix aqueous extract, the level of NO and the activities of iNOS and NADPH-diaphorase were suppressed in a dose-dependent manner, while cell viability increased (Table IV). Analysis of the active components of Sanguisorbae Radix showed the result that its main components are sanguiin H-6 and sanguiin H-11, with small amounts of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, eugeniin and condensed polymeric proanthocyanidin (Fig. 1). Of these five components, sanguiin H-6 exerted the strongest protective activity against NO production, the activities of iNOS and NADPH-diaphorase and cell viability, followed by 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose and sanguiin H-11 (Table IV). This anti-NO activity was comparable to the effect of aminoguanidine, a specific inhibitor of iNOS. Therefore, it was apparent that the NO production-suppressing action of Sanguisorbae Radix is attributable to these polyphenol components.

2.3. Effects of sanguiin H-6 on NO production: Sanguiin H-6 exerted protective activity by reducing NO production by LPS-activated macrophages. It inhibited the expression of iNOS mRNA as well as iNOS activity in a dose-dependent manner, demonstrating for the first time that this compound can inhibit iNOS activity through the regulation of iNOS at the mRNA level (Fig. 2 and Table V). However, it remains unclear whether sanguiin H-6 inhibits the induction of iNOS mRNA by a direct action on LPS, or acts indirectly through the production/release of cytokines, where it could act on the signal transduction pathways involved in cytokine production by tyrosine kinases, or alternatively, whether it inhibits the phosphorylation of proteins induced by the

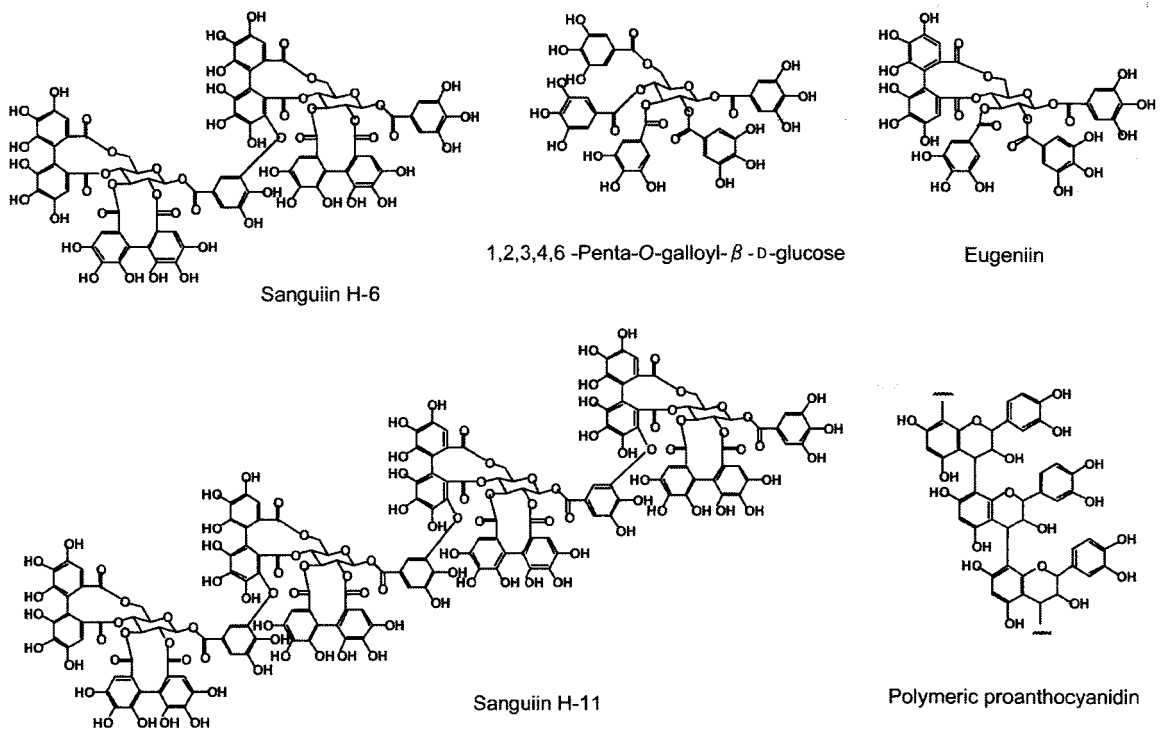

Fig. 1 Chemical structures of components isolated from Sanguisorbae Radix.

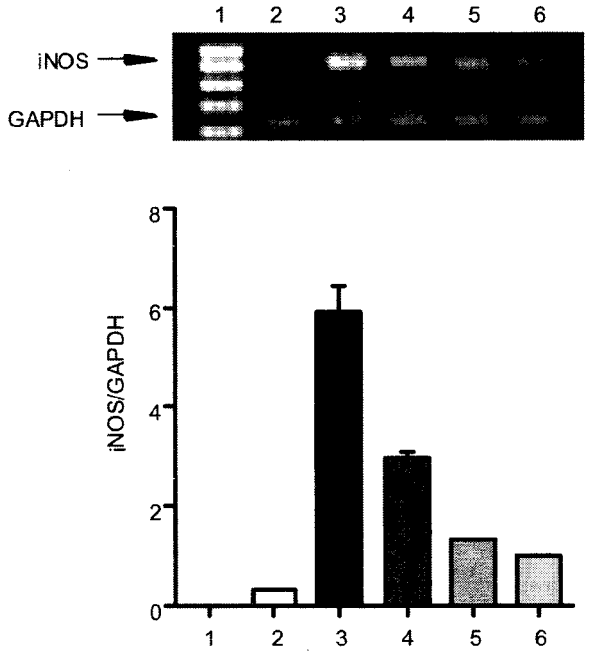

Fig. 2 Effect of sanguiin H-6 on iNOS mRNA expression in activated macrophages. 1, 50 bp marker DNA; 2, none treatment (control); 3, LPS-treated control; 4, LPS-treated sanguiin H-6 (12.5 μM); 5, LPS-treated sanguiin H-6 (25 μM); 6, LPS-treated sanguiin H-6 (50 μM).

Table V Effect of sanguiin H-6 on iNOS activity.

Group	iNOS (pmol/mg protein/min)
None	5.87 ± 0.96
LPS-treatment	
Control	25.98 ± 3.65[b]
Sanguiin H-6 (12.5 μM)	19.98 ± 2.72[b,c]
Sanguiin H-6 (25 μM)	9.80 ± 0.75[a,c]
Sanguiin H-6 (50 μM)	7.01 ± 1.10[c]
Aminoguanidine (50 μM)	9.75 ± 0.61[a,c]

[a]$p<0.05$, [b]$p<0.001$ *vs.* none treatment values, [c]$p<0.001$ *vs.* LPS-treatment control values.

Table VI Effect of sanguiin H-6 on NO production in macrophages.

Group	Nitrite (μM)	Cell viability (%)
None	4.55 ± 0.34	100.0 ± 1.3
LPS-treatment		
Control	49.86 ± 1.44[c]	74.9 ± 2.4[c]
Sanguiin H-6 (12.5 μM)	15.60 ± 0.50[c,e]	82.9 ± 3.6[c,d]
Sanguiin H-6 (25 μM)	12.08 ± 0.96[c,e]	94.7 ± 1.3[a,e]
Sanguiin H-6 (50 μM)	7.75 ± 0.49[c,e]	107.6 ± 3.9[b,e]
Aminoguanidine (50 μM)	11.72 ± 0.53[c,e]	76.0 ± 2.7[c]

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ *vs.* none treatment values, [d]$p<0.01$, [e]$p<0.001$ *vs.* LPS-treatment control values.

cytokines themselves. Although the expression of iNOS mRNA and the iNOS activity were suppressed more as the concentration of sanguiin H-6 increased, the production of NO was suppressed markedly even by a low concentration of this agent, suggesting that sanguiin H-6 directly eliminated NO (Table VI). In another experiment using the NO donor sodium nitroprusside, sanguiin H-6, even at a low concentration, was found to eliminate NO (data not shown). These findings suggest that sanguiin H-6 has the capacity to eliminate NO and suppress NO generation by regulation of iNOS at the mRNA level.

Sanguiin H-6, at a concentration of 25 μM, showed an effect equivalent to that of 50 μM aminoguanidine (Table VI). Aminoguanidine resulted in no improvement in the cell viability, which decreased in the presence of LPS, whereas sanguiin H-6 improved the cell viability in a dose-dependent manner, reducing the toxicity of LPS. Various inhibitors of NO or NOS have been used in attempts to improve or attenuate the pathological processes involved in excessive generation of NO, but conflicting results have been obtained. Using isolated renal proximal tubules, Yu *et al.*[39] observed that the NOS inhibitor *N*-nitro-L-arginine methyl ester protected the renal tubular epithelium against hypoxic injury. Weinberg *et al.*[40] demonstrated that oral administration of N^G-monomethyl-L-arginine prevented the development of glomerulonephritis and reduced the intensity of inflammatory arthritis in MRL-*lpr/lpr* mice. In contrast to these beneficial effects, NOS inhibitors have been shown to aggravate renal dysfunction in several *in vivo* models of acute renal failure.[41,42] Moncada *et al.*[43] have shown that the iNOS expressed in inflammatory cells produces a large amount of NO and this not only acts as an effector for the nonspecific defense mechanism, but also possibly damages normal cells, serving as an effector for autocytoclasis in autoimmune disease. Therefore, the ideal NOS inhibitor should not affect the favorable actions of NO and possibly enhance them, but should block the harmful actions specifically. During the past years, extensive research into the development of ideal NO inhibitors has been performed. Lots of NOS inhibitors demonstrated excellent inhibition of iNOS activity, but they have rarely been used in clinics because the problems of their numerous other effects, such as side and toxic effects, remain to be solved. However, many natural plants and compounds have been found to be highly active inhibitors of iNOS activity and NO scavengers, suggesting that natural plants may be potential sources of NO inhibitors. Currently, the available findings on sanguiin H-6 suggest that this agent has such ideal activity. Although the exact mechanism of action has not been fully elucidated, it may be a promising approach for the development of a safe selective iNOS inhibitor. From these results, Sanguisorbae Radix and its active component sanguiin H-6 would be expected to ameliorate renal injury induced by excessive NO.

3. Protective activity of the Chinese prescription Wen-Pi-Tang against $ONOO^-$-induced renal injury

Wen-Pi-Tang, a Chinese prescription composed of Rhei Rhizoma, Ginseng Radix, Aconiti Tuber, Zingiberis Rhizoma and Glycyrrhizae Radix, is known to enhance cellular defense mechanisms and eliminate impurities accumulated in the body. In particular, it is one of the traditional prescriptions used clinically as a medicine to treat renal failure. To establish experimentally the scientific basis for the actions of Wen-Pi-Tang, whose clinical efficacy is already recognized, we investigated the effects of Wen-Pi-Tang and its component crude drugs using *in vivo* and *in vitro* evaluation systems.[44-65] The present review focuses on the protective activities of Wen-Pi-Tang against $ONOO^-$-induced renal oxidative damage.

3.1. Effects in an *in vitro* $ONOO^-$-generation system : We reported that Wen-Pi-Tang and its component crude drugs caused a significant and concentration-dependent decrease in $ONOO^-$ formation from 3-morpholinosydnonimine (SIN-1) and showed strong $ONOO^-$-scavenging activity.[62] In a cellular system, the protective effect of Wen-Pi-Tang extract against $ONOO^-$-induced renal injury was investigated using renal tubular LLC-PK_1 cells, as renal tubular cells are the most vulnerable target in renal tissue to oxidative stress (Fig. 3). Proximal tubular epithelial cell death was observed under various pathological conditions of chronic renal failure.[66,67] Our results also revealed that exposure to 800 μM SIN-1 resulted in remarkable increases in cellular $ONOO^-$ levels and apoptotic cell death assessed by a

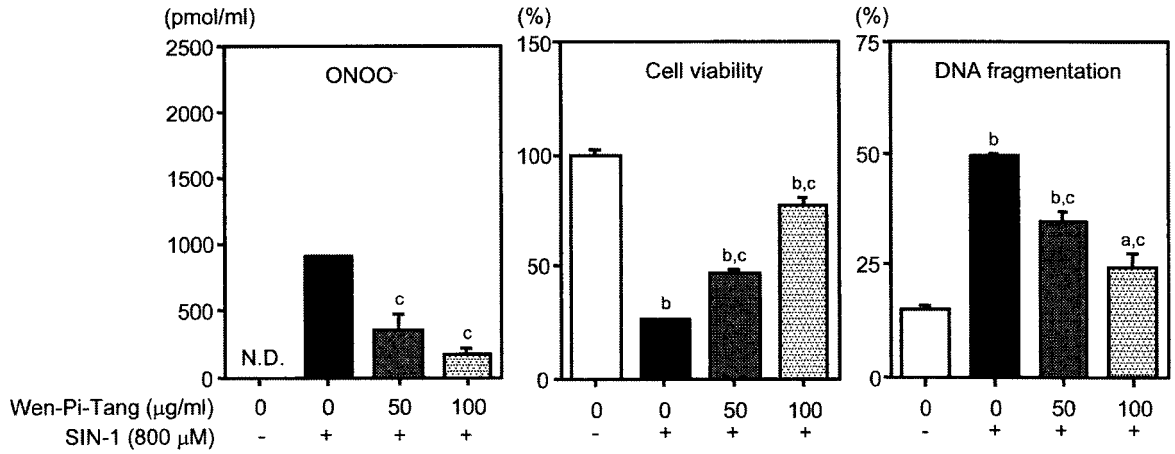

Fig. 3 Effect of Wen-Pi-Tang extract on cellular $ONOO^-$ formation, cell viability and DNA fragmentation in renal tubular LLC-PK_1 cells treated with Wen-Pi-Tang extract together with SIN-1. N.D., not detectable. [a]$p<0.01$, [b]$p<0.001$ *vs.* none treatment values, [c]$p<0.001$ *vs.* SIN-1 treatment values.

Table VII Effect of Wen-Pi-Tang extract on urea nitrogen and Cr levels in serum.

Group	Urea nitrogen (mg/dl)	Cr (mg/dl)
Sham treatment	15.2 ± 1.2	0.33 ± 0.04
LPS plus ischemia-reperfusion		
Control	65.4 ± 0.1[a]	1.59 ± 0.05[a]
Wen-Pi-Tang extract (62.5 mg/kg B.W./day)	51.9 ± 2.6[a,b]	1.27 ± 0.07[a,b]
Wen-Pi-Tang extract (125 mg/kg B.W./day)	48.8 ± 1.9[a,b]	1.20 ± 0.05[a,b]

[a]$p<0.001$ *vs.* sham treatment values, [b]$p<0.001$ *vs.* LPS plus ischemia-reperfusion control values.

DNA fragmentation assay (Fig. 3). Therefore, the formation of $ONOO^-$ by SIN-1 clearly leads to renal cell damage. However, treatment with Wen-Pi-Tang extract, at concentrations of 50 and 100 μg/ml, together with SIN-1 protected renal tubular cells against $ONOO^-$ through scavenging $ONOO^-$ and inhibiting apoptotic cell death in a concentration-dependent manner. Furthermore, the addition of Wen-Pi-Tang extract with SIN-1 attenuated the apoptotic morphological changes and regulated the cell cycle disturbance caused by $ONOO^-$ through G_2/M phase arrest (data not shown). Thus, our results offer the possibility that the potential of Wen-Pi-Tang extract for protection against renal tubular injury is closely involved with $ONOO^-$ formation. Moreover, under the different experimental conditions of the cell culture system, treatment with Wen-Pi-Tang extract both before and after exposure to SIN-1 showed protective activities: reduction of cellular $ONOO^-$ levels, increased cell viability and a decrease in the DNA fragmentation rate (data not shown). Therefore, Wen-Pi-Tang would be expected to both prevent and treat renal injury.

3.2. Effects in an animal model of LPS plus ischemia-reperfusion : On the basis of studies that demonstrated that Wen-Pi-Tang had a protective action on the impaired kidney under oxidative stress as well as free radical-scavenging activity in $ONOO^-$, NO and O_2^- generation systems *in vitro*, Wen-Pi-Tang would be expected to ameliorate renal damage induced by $ONOO^-$ *in vivo*. Therefore, to investigate the effects of Wen-Pi-Tang extract *in vivo* we employed a LPS plus ischemia-reperfusion animal model in which simultaneous and excessive generation of NO and O_2^- occurs and eventually leads to the formation of enough $ONOO^-$ to evaluate its toxicity under the conditions of $ONOO^-$-induced renal failure.[68] The oxidative stress caused by the generation of $ONOO^-$ accompanies acute renal ischemia and contributes to the pathophysiology of renal damage. We found that urea nitrogen and Cr levels in serum were increased by LPS plus ischemia-reperfusion (Table VII), indicating that renal damage and dysfunction resulted from this process. However, Wen-Pi-Tang extract reduced these levels, implying that it ameliorated the renal dysfunction induced by the $ONOO^-$ produced by this process.

$ONOO^-$ in biological fluids can be detected by identifying nitrated tyrosine as a marker of $ONOO^-$ forma-

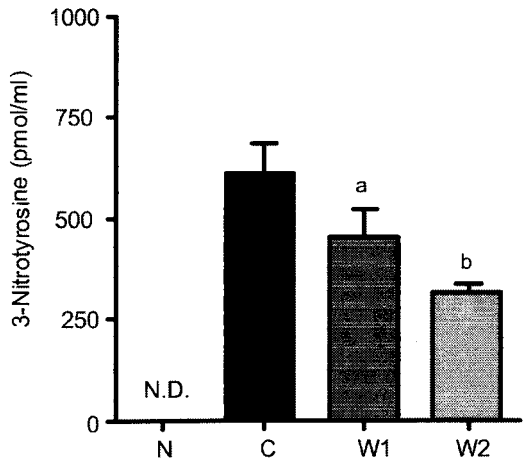

Fig. 4 Effect of Wen-Pi-Tang extract on 3-nitrotyrosine level in plasma. N, sham treatment; C, LPS plus ischemic-reperfused control; W1, LPS plus ischemic-reperfused Wen-Pi-Tang extract (62.5 mg/kg body weight/day); W2, LPS plus ischemic-reperfused Wen-Pi-Tang extract (125 mg/kg body weight/day). [a]$p<0.05$, [b]$p<0.001$ *vs.* LPS plus ischemic-reperfused control values.

tion *in vivo* or a stable end-product of $ONOO^-$ oxidation. The formation of 3-nitrotyrosine in human tissues and animal models of various diseases is a remarkable observation, since nitration has been observed to be a chemical modification that can be used to investigate the functional roles of tyrosine residues in enzymatic activity and protein function.[69] Recently, high levels of 3-nitrotyrosine have been found in the plasma of patients with chronic renal failure,[70] rheumatoid arthritis[71] and septic shock,[72] whereas 3-nitrotyrosine is generally not detectable in the plasma of healthy subjects.[70-72] Noiri *et al.*[73] observed that suppression or scavenging of $ONOO^-$ in ischemic acute renal failure improved renal function, consequently preventing lipid peroxidation and oxidative DNA damage. In our study, the significant increase in 3-nitrotyrosine levels caused by the pathological process of LPS plus ischemia-reperfusion declined after the oral administration of Wen-Pi-Tang extract prior to the process (Fig. 4). Therefore, our results suggest that Wen-Pi-Tang extract would ameliorate $ONOO^-$-mediated renal damage by inhibiting $ONOO^-$ generation.

$ONOO^-$ decomposes to generate a potent oxidant, ·OH, which may cross cell membranes through anion channels and be more toxic to tissues than $ONOO^-$. Therefore, to investigate the formation of ·OH resulting from the decomposition of $ONOO^-$, we measured the levels of tyrosine isomers such as *o*-, *m*- and *p*-tyrosine. Our results revealed that the high levels of tyrosine isomers produced by hydroxylation under the conditions of an $ONOO^-$ generation system *in vivo* were reduced by Wen-Pi-Tang extract (Table VIII). Moreover, the ·OH-scavenging activity of Wen-Pi-Tang extract was confirmed by electron spin resonance analysis of kidney homogenates subjected to the Fenton reaction (data not shown). These findings provide direct evidence that Wen-Pi-Tang extract modulates the generation of $ONOO^-$ and ·OH as secondary reactive end-products stimulated by LPS plus ischemia-reperfusion. Such a protective effect against $ONOO^-$ and ·OH may play an important role in preventing and reversing oxidative damage of tissue and improving renal function.

The major sources of NO and O_2^-, the precursors of $ONOO^-$, are iNOS and xanthine oxidase (XOD), respectively. The activity of iNOS was elevated in the LPS plus ischemia-reperfusion control group compared with that of rats subjected to a sham operation, but the XOD activities of these two groups were not significantly different (Fig. 5). Several studies have shown that although XOD activity initially increased during ischemia, a decline in XOD activity occurred during reperfusion-associated $ONOO^-$ generation, suggesting that $ONOO^-$ could feed back and inhibit XOD.[74-76] Wen-Pi-Tang extract inhibited neither iNOS nor XOD activity (Fig. 5), whereas it inhibited $ONOO^-$ formation (Fig. 4), which

Table VIII Effect of Wen-Pi-Tang extract on *o*-, *m*-, *p*-tyrosine and phenylalanine levels in plasma.

Group	Tyrosine (nmol/ml)			Phenylalanine (nmol/ml)
	o-	*m*-	*p*-	
Sham treatment	91.2 ± 6.9	15.2 ± 0.6	5896 ± 312	8756 ± 352
LPS plus ischemia-reperfusion				
Control	217.9 ± 9.1[c]	39.9 ± 3.5[c]	6730 ± 460[a]	6218 ± 483[b]
Wen-Pi-Tang extract (62.5 mg/kg B.W./day)	217.1 ± 43.6[c]	25.7 ± 3.6[b,d]	6733 ± 396[a]	7746 ± 760
Wen-Pi-Tang extract (125 mg/kg B.W./day)	232.6 ± 41.4[c]	25.7 ± 6.9[b,d]	6431 ± 285	9225 ± 1798[d]

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ *vs.* sham treatment values, [d]$p<0.001$ *vs.* LPS plus ischemia-reperfusion control values.

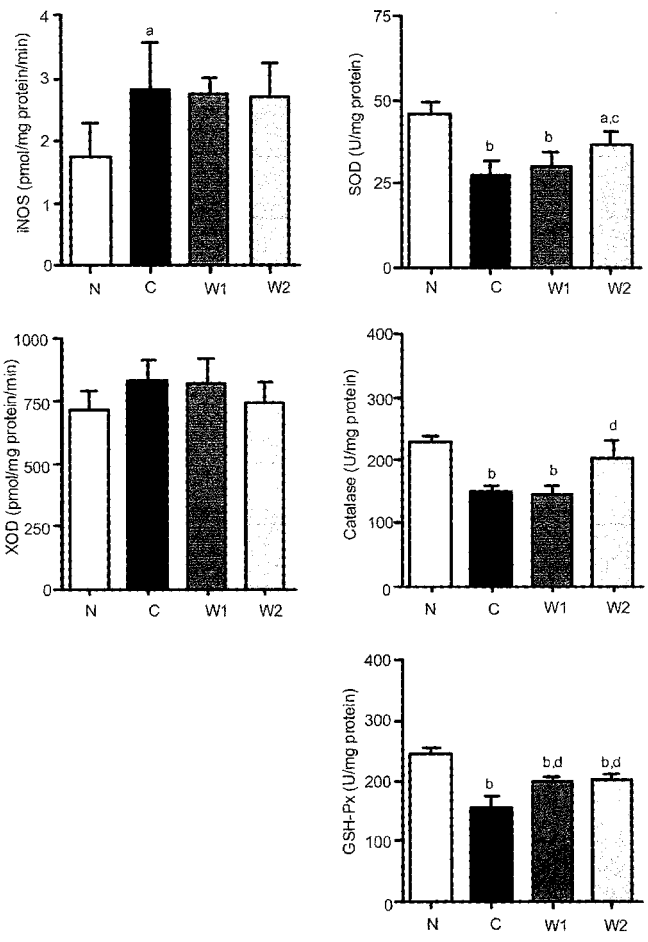

Fig. 5 Effect of Wen-Pi-Tang extract on iNOS, XOD, and radical scavenging enzyme activities in renal tissue. N, sham treatment; C, LPS plus ischemic-reperfused control; W1, LPS plus ischemic-reperfused Wen-Pi-Tang extract (62.5 mg/kg body weight/day); W2, LPS plus ischemic-reperfused Wen-Pi-Tang extract (125 mg/kg body weight/day). $^{a}p<0.05$, $^{b}p<0.001$ *vs.* sham treatment values, $^{c}p<0.01$, $^{d}p<0.001$ *vs.* LPS plus ischemic-reperfused control values.

suggests that the protective property of Wen-Pi-Tang extract was attributable not to the inhibition of NO and O_2^- but to direct scavenging of $ONOO^-$ and ·OH, both of which were involved in the development of oxidative injury and renal dysfunction.

NO has been shown to inhibit catalase and glutathione peroxidase (GSH-Px), which might lead to the elevation of hydrogen peroxide levels and a subsequent increase in $ONOO^-$ production.[77,78] In addition, $ONOO^-$ itself inhibits these enzymes. There is a requirement for cellular defense against excessive $ONOO^-$ generation to protect against oxidative damage. Our results showed that the activities of superoxide dismutase (SOD), catalase and GSH-Px in renal tissue were all significantly suppressed by LPS plus ischemia-reperfusion, which resulted in marked $ONOO^-$ generation (Fig. 5). However, these enzyme activities were effectively increased by the administration of Wen-Pi-Tang extract. This result demonstrates that the destroyed defense system against excessive $ONOO^-$ recovered after the administration of Wen-Pi-Tang extract, resulting in amelioration of the pathological condition induced by $ONOO^-$. In the light of the results of this study, Wen-Pi-Tang would be expected to be a therapeutic agent for $ONOO^-$-associated pathological renal conditions.

3.3. Protective activity of (-)-epicatechin 3-*O*-gallate (ECg) against $ONOO^-$-mediated renal damage: We demonstrated that the most active crude drug ingredient of Wen-Pi-Tang for improving metabolism under conditions of renal failure is Rhei Rhizoma and its beneficial antioxidative effect is mainly attributable to ECg.[45,49,52,55] In the LPS plus ischemia-reperfusion animal model, oral administration of ECg prior to the process attenuated the renal injury induced by $ONOO^-$ through inhibition of lipid peroxidation and enhancement of the biological defence system. In addition, ECg decreased $ONOO^-$ production, but the significant elevation of NO production caused by the LPS plus ischemia-reperfusion process was not suppressed by ECg.[79] Therefore, ECg was considered to act as a specific and direct inhibitor of $ONOO^-$ generation *in vivo* and its action can lead to the improvement of $ONOO^-$-mediated renal failure.

To elucidate the protective mechanisms of ECg against $ONOO^-$, we employed the LLC-PK_1 renal tubular epithelial cell line, as damage to renal tubular epithelial cells has attracted considerable attention as a contributor to renal injury and dysfunction. In addition, SIN-1, an $ONOO^-$ donor, was employed to induce the simultaneous generation of NO and O_2^-. The results of this investigation showed that exposing LLC-PK_1 cells to SIN-1 resulted in significantly reduced cell viability and high $ONOO^-$ production (Fig. 6).[79] However, treatment with ECg before exposure to SIN-1 decreased the formation of $ONOO^-$ without affecting NO levels (data on NO levels not shown) and increased cell survival in a concentration-dependent manner. These results suggest that ECg exerts protective activities in a cellular $ONOO^-$ generation system through scavenging $ONOO^-$ and

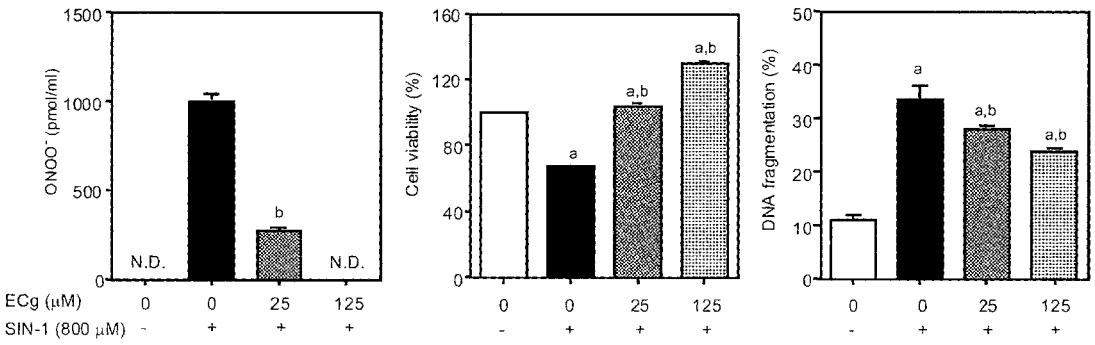

Fig. 6 Effect of epicatechin 3-*O*-gallate on SIN-1-induced $ONOO^-$ formation, cell viability and DNA fragmentation in renal epithelial cells, LLC-PK$_1$. N.D., not detectable. $^a p<0.001$ *vs.* none treatment values, $^b p<0.001$ *vs.* SIN-1 treatment values.

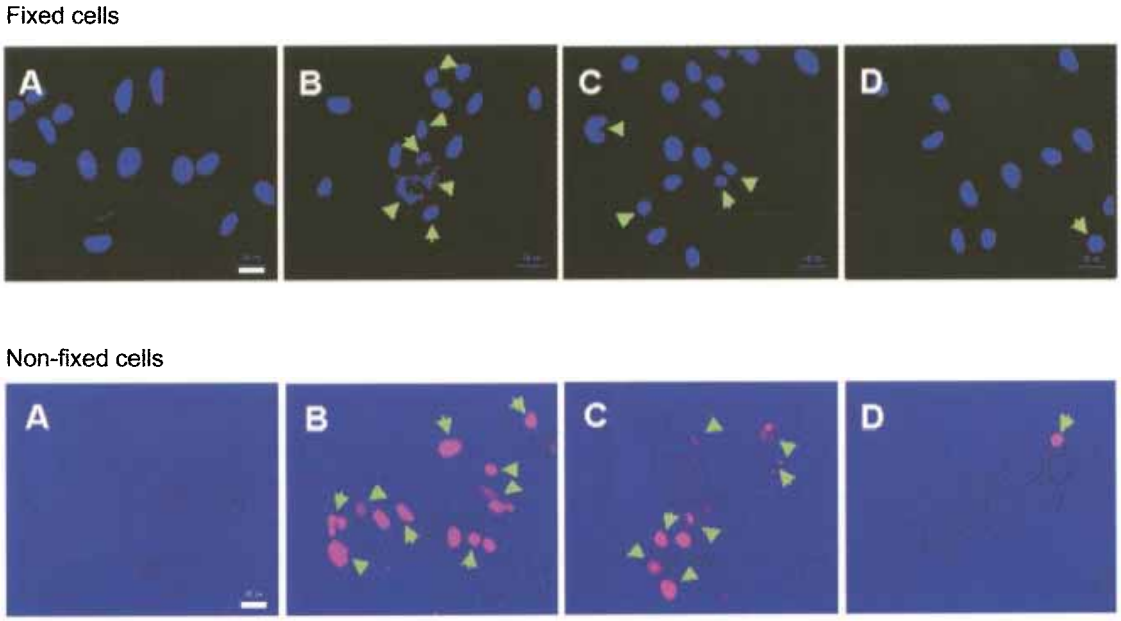

Fig. 7 Morphological changes of fixed (upper panel) and non-fixed (lower panel) cells. After incubation with epicatechin 3-*O*-gallate for 24 h, SIN-1 was added and the cells were incubated for a further 4 h. A, none treatment; B, SIN-1 (800 μM) treatment; C, SIN-1 (800 μM) and epicatechin 3-*O*-gallate (25 μM) treatment; D, SIN-1 (800 μM) and epicatechin 3-*O*-gallate (125 μM) treatment. Arrows indicate apoptotic cells. Magnification, x 800. Bar represents 20 μm.

inhibiting cell death caused by $ONOO^-$.

The cytotoxic effects of $ONOO^-$ have been ascribed to DNA damage, inhibition of DNA repair and induction of cell death either by apoptosis or necrosis.[80-83] In particular, apoptosis has been regarded to contribute to extensive cell loss in many pathological states. Moreover, the oxidative stress resulting from free radicals disturbed the cell cycle, eventually inhibiting cell proliferation.[84,85] Most organisms respond to biological damage by regulating the cell cycle, cell proliferation by apoptosis and the DNA repair pathway. Exposure of LLC-PK$_1$ cells to SIN-1 caused apoptotic cell death, reflected by DNA fragmentation, and morphological changes, such as small and nuclear fragmentation (Figs. 6 and 7). In addition, $ONOO^-$ generated by SIN-1 disturbed the cell cycle by decreasing the G_2/M cell ratio (Table IX). However, the presence of ECg prior to SIN-1 exposure resulted in decreases in the DNA fragmentation rate and characteristic apoptotic morphological changes, and regulated the cell cycle by promoting G_2/M phase arrest.[79] These results indicate that the protective activity of ECg against SIN-1 involved decreases in apoptosis-mediated cell death and regulation of the cell cycle.

Table IX Effect of (-)-epicatechin 3-*O*-gallate on the cell cycle.

Treatment	Percentage of cells in each phase of cell cycle (%)		
	G_0/G_1	S	G_2/M
None	61.1 ± 3.0	32.3 ± 1.9	6.7 ± 1.1
SIN-1 (800 μM)	64.4 ± 0.6	34.5 ± 1.6	1.2 ± 1.2[c]
SIN-1 (800 μM) and epicatechin 3-*O*-gallate (25 μM)	60.9 ± 1.9	36.7 ± 2.7[a]	2.4 ± 0.7[b]
SIN-1 (800 μM) and epicatechin 3-*O*-gallate (125 μM)	63.4 ± 1.8	30.9 ± 1.2	5.7 ± 0.8[d]

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ *vs.* none treatment values, [d]$p<0.01$ *vs.* SIN-1 treatment values.

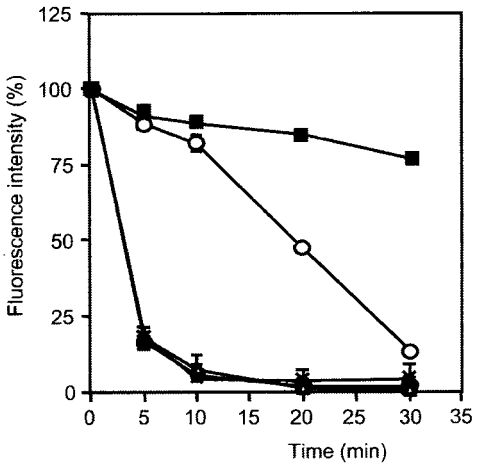

Fig. 8 Time response curve of green tea extract (○), polyphenols (■), caffeine (*), and theanine (△) at 1 μg/ml, and non-additive control (●) on allophycocyanin quenching induced by AAPH.

4. Activity of green tea against renal oxidative damage

Green tea contains low-molecular-weight polyphenols belonging to the flavan-3-ol class of flavonoids that possess considerable antioxidative activities. Furthermore, the antioxidative activity of green tea was found to contribute to the inhibition of hypertension, mutagenesis and tumorigenesis and to protect against renal diseases in several experimental systems *in vitro* and *in vivo*.[86-89] The present review summarizes the antioxidative activities of green tea and its polyphenols on the basis of experiments *in vitro* and *in vivo* and the results of clinical trials.

4.1. Effects on free radical- and glucose-mediated protein damage : In our recent study, we demonstrated that green tea polyphenols exerted protective activity against protein oxidation and glycation.[90] Protein oxidative damage is directly involved in the pathogenesis of many diseases. Free radicals can induce protein modifications, including loss of protein function, such as the activities of enzymes, receptors, and membrane transporters, in turn resulting in biological dysfunction.[91,92] To examine the protective effect of green tea against protein oxidation induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), we measured the fluorescence intensity of allophycocyanin, a protein with natural fluorescence. Following treatment with AAPH, its intrinsic fluorescence was rapidly diminished, reflecting oxidation of allophycocyanin. However, of the components of green tea, the polyphenols proved to be the most potent against AAPH-induced protein damage (Fig. 8), whereas caffeine and theanine were found to have relatively weak activities. It is known that the free radicals generated from AAPH react with oxygen molecules rapidly to yield peroxyl radicals. Therefore, it can be assumed that the free radicals related to protein damage in this study were peroxyl radicals, and that the peroxyl radical-scavenging property of green tea polyphenols plays an important role in protection against free radical-mediated protein damage.

Proteins in the body are also modified by glucose through the glycation reaction. This reaction finally produces advanced glycation end products (AGEs) and the accumulation of AGEs has been observed under the pathological conditions of oxidative stress-induced diseases.[93-95] Oxidative reactions participate extensively in the process of AGEs formation,[96,97] indicating that biological proteins are susceptible to modification *in vivo* by AGEs under conditions of oxidative stress. The contribution of AGEs to some pathological conditions, including diabetic complications, aging and Alzheimer's disease, has attracted considerable interest in recent years. In addition, it has been reported that antioxidant and radical scavengers inhibit these processes. Green tea extract and its polyphenols inhibited AGEs formation significantly, whereas caffeine and theanine showed

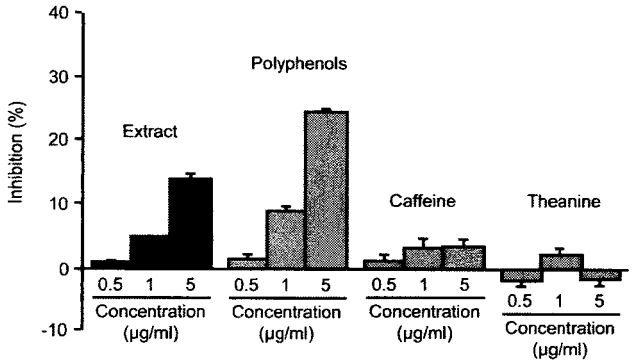

Fig. 9 Effect of green tea extract and its components on AGEs formation.

Table X Effect of green tea extract and polyphenols on viability and thiobarbituric acid-reactive substances of cells treated with AAPH.

Material	Concentration (μg/ml)	Cell viability (%)	Thiobarbituric acid-reactive substances (nmol/well)
Extract	0	65.6 ± 1.1[b]	0.131 ± 0.004[b]
	5	72.3 ± 1.1[b,c]	0.125 ± 0.013[b]
	25	79.2 ± 4.8[b,e]	0.108 ± 0.005[a,d]
	50	80.7 ± 3.7[b,e]	0.101 ± 0.008[e]
Polyphenols	0	65.6 ± 1.1[b]	0.131 ± 0.004[b]
	5	71.8 ± 2.0[b,c]	0.110 ± 0.008[b,e]
	25	87.1 ± 5.1[b,e]	0.091 ± 0.003[e]
	50	87.9 ± 2.6[b,e]	0.084 ± 0.001[a,e]
	-	100.0 ± 1.0	0.093 ± 0.003

[a]$p<0.05$, [b]$p<0.001$ *vs.* AAPH none treatment values, [c]$p<0.05$, [d]$p<0.01$, [e]$p<0.001$ *vs.* AAPH treatment values.

weak activity (Fig. 9), suggesting a potential role for green tea polyphenols in the treatment of oxidative stress-induced diseases.

4.2. Antioxidative activity against AAPH in a cellular system : To evaluate the antioxidative properties of green tea in a cellular system, we employed an AAPH model system with LLC-PK$_1$ renal tubular epithelial cells.[98] Several studies demonstrated that AAPH decreased the viability of hepatic cells, neurons and aortic endothelial cells, induced apoptosis of these cells and resulted in loss of viability.[99-101] In this study, we demonstrated clearly that AAPH also led to decreased viability of LLC-PK$_1$ cells and increased formation of thiobarbituric acid-reactive substances (Table X), indicating that LLC-PK$_1$ cells sustained free radical damage caused by AAPH. Terao and Niki[102] reported that there are three types of organ or tissue damage induced by AAPH. The most striking structural changes following the administration of AAPH include degeneration, swelling and disruption of the capillary endothelial cells in various organs. The second type is death of lymphocytes in the lymphoid tissues and the third type of AAPH intoxication is characterized by marked fatty degeneration of the kidneys and liver. Although it is not completely understood which types of damage are involved in AAPH-induced LLC-PK$_1$ cellular injury, we hypothesize that AAPH treatment leads to the degeneration, disruption and death of LLC-PK$_1$ cells. However, green tea extract, and its polyphenols protected against AAPH-induced cellular damage by inhibiting cellular loss and lipid peroxidation resulting from the peroxyl radicals generated by AAPH (Table X). In AAPH-induced cell injury and peroxidation, scavenging of lipid peroxyl radicals plays a considerable part in antioxidative activity. We suggest that green tea extract and its polyphenols scavenge peroxyl radicals generated from AAPH. In addition, we hypothesize that they might protect the renal cell against free radicals by either one or a combination of the following mechanisms. First, they may act as a chelator to inactivate catalytic cations involved in the initiation of free radicals. Second, they may function as a free-radical chain reaction interrupter by trapping the free radicals generated by AAPH.

4.3. Protective activity in rats with renal failure: The antioxidative activity of green tea polyphenols against renal injury *in vivo* was also confirmed.[103] The effect was examined in nephrectomized rats, a widely used animal model for investigating the progression of glomerular disorders. The increases in serum urea nitrogen and Cr levels in nephrectomized rats were suppressed by green tea polyphenols (Table XI). The removal of uremic toxins that affect renal function would have a beneficial effect by inhibiting glomerular deterioration through blocking the uremic toxin-associated vicious cycle that results in renal failure. In addition, the decrease in the creatinine clearance (Ccr) value under conditions of renal failure was significantly reversed after the administration of green tea polyphenols (Table XI), suggesting that they would contribute to the improvement of glomerular filtration.

The animal model of renal failure produced by nephrectomy shows hypertrophy or swelling of the

Table XI Effect of green tea polyphenols on renal function parameters.

Day	Group	Dose (mg/kg B.W./day)	s-Urea nitrogen (mg/dl)	s-Cr (mg/dl)	Ccr (ml/min/kg B.W.)	u-Protein (mg/day)
0	Nephrectomized rats					
	Control	-	42.3 ± 1.4	0.58 ± 0.02	4.18 ± 0.19	34.0 ± 6.3
	Polyphenols	10	43.2 ± 2.9	0.59 ± 0.02	4.11 ± 0.18	32.7 ± 1.9
	Polyphenols	20	41.6 ± 2.5	0.57 ± 0.02	4.21 ± 0.19	36.1 ± 1.2
20	Nephrectomized rats					
	Control	-	39.7 ± 3.5	0.77 ± 0.05	2.73 ± 0.20	33.6 ± 2.7
	Polyphenols	10	28.7 ± 1.3^{c}	0.69 ± 0.02^{b}	3.43 ± 0.18^{c}	25.2 ± 2.3^{b}
	Polyphenols	20	30.5 ± 1.4^{c}	0.59 ± 0.02^{c}	4.22 ± 0.19^{c}	26.7 ± 3.3^{b}
40	Nephrectomized rats					
	Control	-	40.2 ± 3.9	0.84 ± 0.03	2.79 ± 0.23	51.6 ± 6.1
	Polyphenols	10	31.7 ± 1.9^{b}	0.73 ± 0.02^{c}	3.66 ± 0.15^{c}	32.8 ± 4.4^{c}
	Polyphenols	20	32.1 ± 2.2^{b}	0.70 ± 0.02^{c}	3.76 ± 0.10^{c}	32.6 ± 5.3^{c}
60	Nephrectomized rats					
	Control	-	42.8 ± 4.4	0.83 ± 0.05	2.62 ± 0.16	50.4 ± 6.3
	Polyphenols	10	34.0 ± 2.8^{b}	0.74 ± 0.03^{b}	3.00 ± 0.19^{a}	34.3 ± 8.3^{b}
	Polyphenols	20	30.2 ± 1.9^{c}	0.69 ± 0.03^{c}	3.04 ± 0.16^{b}	32.1 ± 4.0^{b}
80	Nephrectomized rats					
	Control	-	51.8 ± 2.7	1.05 ± 0.05	1.91 ± 0.12	51.1 ± 6.4
	Polyphenols	10	36.9 ± 3.0^{c}	0.90 ± 0.05^{b}	2.65 ± 0.13^{c}	29.2 ± 4.7^{c}
	Polyphenols	20	40.2 ± 3.6^{c}	0.86 ± 0.05^{c}	2.80 ± 0.15^{c}	33.8 ± 3.1^{c}
	Normal rats		16.4 ± 0.3	0.48 ± 0.03	5.43 ± 0.48	9.2 ± 0.3

$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ *vs.* nephrectomized control values.

Table XII Effect of green tea polyphenols on the activities of reactive oxygen species-scavenging enzymes in rats after excision of 3/4 of their kidney volume.

Group	Dose (mg/kg B.W./day)	SOD (U/mg protein)	Catalase (U/mg protein)	GSH-Px (U/mg protein)
Nephrectomized rats				
Control	-	8.75 ± 0.40	142.7 ± 11.8	69.63 ± 2.02
Green tea polyphenols	10	10.68 ± 0.48^{b}	213.2 ± 13.9^{c}	71.91 ± 3.41
Green tea polyphenols	20	11.66 ± 0.54^{c}	224.4 ± 10.9^{c}	76.97 ± 3.15^{a}
Normal rats		18.33 ± 1.00	225.9 ± 8.7	85.12 ± 3.95

$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ *vs.* nephrectomized control values.

Table XIII Histopathological evaluation of the kidney.

Parameter	Control	Green tea polyphenols (10 mg)	Green tea polyphenols (20 mg)
Degree of mesangial proliferation			
Normal	0	0	0
Slight	1	3	4
Moderate	4	3	2
Severe	1	0	0
Glomerular sclerosis index	1.59 ± 0.18	1.24 ± 0.12^{a}	1.15 ± 0.13^{a}

$^{a}p<0.01$ *vs.* control values.

Table XIV Effect of green tea polyphenols on serum Cr, MG and the MG/Cr ratio in patients receiving dialysis.

Duration of treatment (month)	Cr (mg/dl)	MG (μg/dl)	MG/Cr ($\times 10^{-3}$)
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]

[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ *vs.* pre-treatment values.

Table XV Effect of green tea polyphenols on serum β_2-MG in patients receiving dialysis.

Duration of treatment (month)	β_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]

[a]$p<0.01$, [b]$p<0.001$ *vs.* pre-treatment values.

remaining kidney with an increase in its weight (data not shown). On the basis of the fact that the remaining kidney shows significantly increased oxygen consumption and enhanced ATP synthesis, Schrier *et al.*[104] and Harris *et al.*[105] suggested that free radicals are involved in various ways in the occurrence and progression of renal failure. The evaluation of antioxidative enzymes revealed significant decreases in the activities of SOD, catalase and GSH-Px (Table XII), indicating that the free radical-scavenging system was destroyed in nephrectomized rats. However, green tea polyphenols enhanced the antioxidative defense system through the elevation of SOD, catalase and GSH-Px activities.

To analyze the effects of green tea polyphenols on renal tissue lesions, we focused on the degree of mesangial proliferation, which revealed that renal failure was advanced in nephrectomized rats (Table XIII). It was suggested that following subtotal nephrectomy, some growth factor induces glomerular hypertrophy and mesangial proliferation, the former leading to a disorder in the glomerular basement membrane or epithelial cells, resulting in protein leakage, and the latter leading to glomerular sclerosis. We found that green tea polyphenols suppressed the leakage of urinary protein (Table XI), suggesting that they delayed the progression of glomerular hypertrophy. In addition, oral administration of green tea polyphenols to nephrectomized rats inhibited mesangial proliferation (Table XIII), suggesting the green tea polyphenols protected against renal lesion development. This *in vivo* study indicates the antioxidative properties of green tea polyphenols protect against renal injury.

4.4. The role of green tea polyphenols in dialysis patients : On the basis of several *in vitro* and *in vivo* studies on the antioxidative activities of green tea and its polyphenol compounds, we administered green tea polyphenols to dialysis patients under excessive oxidative conditions and evaluated the usefulness of these compounds in their treatment.[106] As shown in Table XIV, the serum level of Cr decreased significantly after 3 months of green tea polyphenols administration, and this effect was maintained until the end of the 6-month administration period. Moreover, the methylguanidine (MG) level was reduced significantly after one month of green tea polyphenols administration. Cr is frequently used in the clinical setting as a renal function parameter and it is a precursor in the conversion of creatol to MG. In a previous study, we 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 one and related to the generation of ·OH.[107-112] Since the determination of these components is useful in evaluating the pathological features of renal failure, we measured the changes in the serum levels of Cr and MG in chronic renal failure patients to assess the antioxidant activity of green tea polyphenols, which were administered to patients undergoing dialysis. This study showed that the administration of green tea polyphenols led to reductions in the serum levels of Cr and MG and the MG/Cr ratio, which suggests that green tea polyphenols would scavenge ·OH and the improvement of renal dysfunction would be attributable to this radical-scavenging activity.

Reduction of the β_2-microglobulin (β_2-MG) level is desirable in order to prevent the complications associated

with prolonged dialysis, including amyloidosis. Green tea polyphenols caused a significant decrease at every measurement point during the 6-month administration period, except at 2 months after the start of administration, as shown in Table XV. When the suppressive effect was analyzed in three groups of patients classified according to their MG levels at the baseline (i.e., according to the severity of oxidative stress), a significant fall in β_2-MG was found in the high β_2-MG group during the green tea polyphenols administration period. It was notable that this decrease in β_2-MG occurred despite the use of non-high-performance dialysis, with which it is difficult to eliminate β_2-MG. In addition, green tea polyphenols ameliorated pain in the hip, cubitus, coax and fingers of the dialysis patients, suggesting that green tea polyphenols inhibited the deposition of β_2-MG in tissue. Furthermore, there were no significant changes in blood pressure, other general laboratory parameters or subjective symptoms during the green tea polyphenols administration period (data not shown). This clinical study supports the results of *in vitro* and *in vivo* studies on the antioxidative activities of green tea and its polyphenols, and indicates that they may be potential novel treatments for renal injury.

It has also been suggested that structural specificity is involved in the manifestation of the antioxidative activity of green tea polyphenols.[113-117] (-)-Epigallocatechin 3-*O*-gallate (EGCg), (-)-gallocatechin 3-*O*-gallate and ECg had stronger activities than gallate free polyphenols against AAPH-induced protein oxidation, AGEs formation and cellular damage caused by AAPH.[90,98] These findings indicate that the *O*-dihydroxy structure at the 5' position in the B ring and the galloyl groups at the 3 position play important roles in the protective activity of green tea polyphenols. In particular, EGCg exerted the most marked cellular protective activity against AAPH. Moreover, EGCg accounts for the largest fraction of the components of green tea polyphenols. Taking this fact into consideration, the antioxidative activity of green tea polyphenols would appear to be mainly ascribable to EGCg. Several studies also showed that EGCg is stronger than any other catechin in providing protection against oxidation.[118,119] The antioxidative potential of green tea polyphenols is worthy of recognition, even though the mechanisms responsible for the activity have not been fully determined. In addition, unlike Sanguisorbae Radix and Wen-Pi-Tang, green tea polyphenols are considered to exhibit antioxidative activity against various kinds of free radicals, including peroxyl radicals and ·OH, and, therefore, may be a more effective therapeutic agent for oxidative stress-induced pathological conditions.

Acknowledgements

The authors thank Drs. Kenichi Kitani, Cui Ping Chen, Hae Young Chung, Kazumasa Aoyagi and Takashi Tanaka for their support of this research. This work was supported, in part, by grants from the Japan Foundation for Aging and Health and the Japan China Medical Association.

和文抄録

酸化ストレスは，腎疾患を含めた種々の疾患に関与していると考えられており，この酸化ストレスを防御することが出来れば，腎疾患の進行やそれに付随した合併症を予防することが出来るのではないかとの期待が高まっている。しかし，合成抗酸化剤は毒性と副作用が危惧され，近年，植物素材からの抗酸化物質が注目されているが，本稿では伝統薬物（地楡，温脾湯）と緑茶について紹介する。地楡とその成分の sanguiin H-6 は，NO 由来の酸化障害に抗酸化因子として腎病態に作用し，温脾湯と (-)-epicatechin 3-*O*-gallate は $ONOO^-$ を消去して，$ONOO^-$ からのフリーラジカルカスケードの産生を断ち切って，腎に好影響をもたらしていた。一方，緑茶ポリフェノールは *in vitro, in vivo* の実験系のいずれにおいても抗酸化効果を発揮するとともに，酸化亢進状態の透析患者に対しその有用性が示唆された。現在に至るまで腎疾患を狙ったフリーラジカル消去剤はおろか，画期的な治療薬すら開発されていないのが現状であるが，フリーラジカルの消去という観点からの新しいアプローチを提唱したい。

*〒930-0194 富山市杉谷 2630
富山医科薬科大学和漢薬研究所 横澤隆子

References

1) Chertow, G.M., Bullard, A. and Lazarus, J.M.: Nutrition and the dialysis prescription. *Nephrology* **16**, 79-89, 1996.
2) Star, R.A.: Treatment of acute renal failure. *Kidney Int.* **54**, 1817-1831, 1998.

3) Dobashi, K., Ghosh, B., Orak, J.K., Singh, I. and Singh, A.K.: Kidney ischemia-reperfusion: modulation of antioxidant defenses. *Mol. Cell. Biochem.* **205**, 1-11, 2000.
4) Galle, J.: Oxidative stress in chronic renal failure. *Nephrol. Dial. Transplant.* **16**, 2135-2137, 2001.
5) Yaqoob, M., McClelland, P., Patrick, A.W., Stevenson, A., Mason, H., White, M.C. and Bell, G.M.: Evidence of oxidant injury and tubular damage in early diabetic nephropathy. *QJM* **87**, 601-607, 1994.
6) Reckelhoff, J.F., Kanji, V., Racusen, L.C., Schmidt, A.M., Yan, S.D., Marrow, J., Roberts, L.J.II. and Salahudeen, A.K.: Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of F_2-isoprostanes in aging kidneys. *Am. J. Physiol.* **274**, R767-R774, 1998.
7) Craig, W.J.: Health-promoting properties of common herbs. *Am. J. Clin. Nutr.* **70** (suppl), 491s-499s, 1999.
8) Zhao, Y., Wang, X., Kawai, M., Liu, J., Liu, M. and Mori, A.: Antioxidant activity of Chinese ant extract preparations. *Acad. Med. Okayama* **49**, 275-279, 1995.
9) Robak, J. and Gryglewski, R.J.: Bioactivity of flavonoids. *Pol. J. Pharmacol.* **48**, 555-564, 1996.
10) Yokozawa, T., Dong, E., Liu, Z.W. and Oura, H.: Antiperoxidation activity of traditional Chinese prescriptions and their main crude drugs *in vitro*. *Nat. Med.* **51**, 92-97, 1997.
11) Yokozawa, T., Dong, E., Liu, Z.W. and Shimizu, M.: Antioxidative activity of flavones and flavonols *in vitro*. *Phytother. Res.* **11**, 446-449, 1997.
12) Yokozawa, T., Chen, C.P. and Liu, Z.W.: Effect of traditional Chinese prescriptions and their main crude drugs on 1,1-diphenyl-2-picrylhydrazyl radical. *Phytother. Res.* **12**, 94-97, 1998.
13) Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G. and Nishioka, I.: Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochem. Pharmacol.* **56**, 213-222, 1998.
14) Mitsuma, T., Yokozawa, T., Oura, H., Terasawa, K. and Narita, M.: Clinical evaluation of Kampo medication, mainly with Wen-Pi-Tang, on the progression of chronic renal failure. *Jpn. J. Nephrol.* **41**, 769-777, 1999.
15) Gariballa, S.E. and Sinclair, A.J.: Nutrition, ageing and ill health. *Br. J. Nutr.* **80**, 7-23, 1998.
16) Katan, M.B.: Functional foods. *Lancet* **354**, 794, 1999.
17) Milner, J.A.: Functional foods: the US perspective. *Am. J. Clin. Nutr.* **71** (suppl), 1654s-1659s, 2000.
18) Sueoka, N., Suganuma, M., Sueoka, E., Okabe, S., Matsuyama, S., Imai, K., Nakachi, K. and Fujiki, H.: A new function of green tea: prevention of lifestyle-related diseases. *Ann. NY. Acad. Sci.* **928**, 274-280, 2001.
19) Kono, S., Shinchi, K., Ikeda, N., Yanai, F. and Imanishi, K.: Green tea consumption and serum lipid profiles: a cross-sectional study in northern Kyushu. *Jpn. Prev. Med.* **21**, 526-531, 1992.
20) Imai, K. and Kakachi, K.: Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *Br. J. Med.* **310**, 693-696, 1995.
21) Kono, S., Shinchi, K., Wakabayashi, K., Honjo, S., Todoroki, I., Sakurai, Y., Imanishi, K., Nishikawa, H., Ogawa, S. and Katsurada, M.: Relation of green tea consumption to serum lipids and lipoproteins in Japanese man. *J. Epidemiol.* **6**, 128-133, 1996.
22) Yokozawa, T., Chen, C.P. and Tanaka, T.: Direct scavenging of nitric oxide by traditional crude drugs. *Phytomedicine* **6**, 453-463, 1999.
23) Chen, C.P., Yokozawa, T. and Kitani, K.: Beneficial effects of Sanguisorbae Radix in renal dysfunction caused by endotoxin *in vivo*. *Biol. Pharm. Bull.* **22**, 1327-1330, 1999.
24) Chen, C.P., Yokozawa, T. and Tanaka, T.: Protective effect of Sanguisorbae Radix against apoptosis and function of renal tissues subjected to ischemia-reperfusion. *J. Trad. Med.* **16**, 97-101, 1999.
25) Yokozawa, T., Chen, C.P., Tanaka, T. and Kitani, K.: A study on the nitric oxide production-suppressing activity of Sanguisorbae Radix components. *Biol. Pharm. Bull.* **23**, 717-722, 2000.
26) Yokozawa, T. and Chen, C.P.: Evidence suggesting a nitric oxide-scavenging activity for traditional crude drugs, and action mechanisms of Sanguisorbae Radix against oxidative stress and aging. *J. Am. Aging Assoc.* **24**, 19-30, 2001.
27) Yokozawa, T., Chen, C.P., Rhyu, D.Y., Tanaka, T., Park, J.C. and Kitani, K.: Potential of sanguiin H-6 against oxidative damage in renal mitochondria and apoptosis mediated by peroxynitrite *in vivo*. *Nephron* **92**, 133-141, 2002.
28) Yokozawa, T., Chen, C.P., Tanaka, T. and Kitani, K.: Effects of sanguiin H-6, a component of Sanguisorbae Radix, on lipopolysaccharide-stimulated nitric oxide production. *Biochem. Pharmacol.* **63**, 853-858, 2002.
29) Yokozawa, T.: Examination of the nitric oxide production-suppressing activity in Sanguisorbae Radix. *Orient. Pharm. Exp. Med.* **2**, 69-79, 2002.
30) Galle, J. and Wanner, C.: Impact of nitric oxide on renal hemodynamics and glomerular function: Modulation by atherogenic lipoproteins? *Kidney Blood Pressure Res.* **19**, 2-15, 1996.
31) Craven, P.A., DeRubertis, F.R. and Melhem, M.: Nitric oxide in diabetic nephropathy. *Kidney Int.* **60**, s46-s53, 1997.
32) Kone, B.C.: Nitric oxide in renal health and disease. *Am. J. Kidney Dis.* **30**, 311-333, 1997.
33) Goliogorsky, M.S. and Noiri, E.: Duality of nitric oxide in acute renal injury. *Semin. Nephrol.* **19**, 263-271, 1999.
34) Kone, B.C. and Baylis, C.: Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am. J. Physiol.* **272**, F561-F578, 1997.
35) Bachmann, S. and Mundel, P.: Nitric oxide in the kidney: synthesis, localization, and function. *Am. J. Kidney Dis.* **24**, 112-129, 1994.
36) Norris, P.J., Charles, I.G., Scorer, C.A. and Emson, P.C.: Studies on the localization and expression of nitric oxide synthase using histochemical techniques. *Histochem. J.* **27**, 745-756, 1995.
37) Mitchell, J.A., Kohlhaas, K.L., Matsumoto, T., Pollock, J.S., Forstermann, U., Warner, T.D., Schmidt, H.H. and Murad, F.: Induction of NADPH-dependent diaphorase and nitric oxide synthase activity in aortic smooth muscle and cultured macrophages. *Mol. Pharmacol.* **41**, 1163-1168, 1992.
38) Tracey, W.R., Nakane, M., Pollock, J.S. and Forstermann, U.: Nitric oxide synthases in neuronal cells, macrophages and endothelium are NADPH diaphorases, but represent only a fraction of total cellular NADPH diaphorase activity. *Biochem. Biophys. Res. Commun.* **195**, 1035-1040, 1993.
39) Yu, L., Gengaro, P.E., Niederberger, M., Burke, T.J. and Schrier, R.W.: Nitric oxide: a mediator in rat tubular hypoxia/reoxygenation injury. *Proc. Natl. Acad. Sci. USA* **91**, 1691-1695, 1994.
40) Weinberg, J.B., Granger, D.L., Pisetsky, D.S., Seldin, M.F., Misukonis, M.A., Mason, S.N., Pippen, A.M., Ruiz, P., Wood, E.R. and Gilkeson, G.S.: The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric

oxide production and nitric oxide synthase expression in MRL-*lpr/lpr* mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered N^G-monomethyl-L-arginine. *J. Exp. Med.* **179**, 651-660, 1994.

41) Schwartz, D., Blum, M., Peer, G., Wollman, Y., Maree, A., Serban, I., Grosskopf, I., Cabili, S., Levo, Y. and Iaina, A.: Role of nitric oxide (EDRF) in radiocontrast acute renal failure in rats. *Am. J. Physiol.* **267**, F374-F379, 1994.
42) Mashiach, E., Sela, S., Winaver, J., Shasha, S.M. and Kristal, B.: Renal ischemia-reperfusion injury: contribution of nitric oxide and renal blood flow. *Nephron* **80**, 458-467, 1998.
43) Moncada, S., Palmer, R.M. and Higgs, E.A.: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43**, 109-142, 1991.
44) Yokozawa, T., Suzuki, N., Zheng, P.D., Oura, H. and Nishioka, I.: Effect of orally administered rhubarb extract in rats with chronic renal failure. *Chem. Pharm. Bull.* **32**, 4506-4513, 1984.
45) Oura, H., Chung, H.Y. and Yokozawa, T.: Effect of each component crude drug of the traditional Chinese prescription "Onpi-to" on rats with chronic renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 351-356, 1985.
46) Zheng, P.D., Yokozawa, T., Oura, H. and Nakada, T.: Effect of orally administered Onpi-to to rats with chronic renal failure on blood flow in renal tissue, blood pressure, and hormone levels in blood. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 37-44, 1986.
47) Yokozawa, T., Zheng, P.D., Mo, Z.L. and Oura, H.: The effect of Onpi-to on urinary excretion of methylguanidine in rats with chronic renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 198-201, 1986.
48) Yokozawa, T., Wu, X.Q., Lee, T.W. and Oura, H.: Onpi-to administration increases renal function in rats with renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 179-183, 1988.
49) Yokozawa, T., Wu, X.Q., Fujioka, K. and Oura, H.: Effects of crude drug extract of Ompi-to on renal function in rats with renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **6**, 64-69, 1989.
50) Yokozawa, T., Wu, X.Q. and Oura, H.: Onpi-to prevents the progression of renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **6**, 147-151, 1989.
51) Yokozawa, T., Wu, X.Q., Lee, T.W. and Oura, H.: Effects of Ompi-to on the urinary levels of prostaglandin and kallikrein in rats with renal failure. *J. Med. Pharm. Soc. WAKAN-YAKU* **6**, 188-192, 1989.
52) Yokozawa, T., Fujioka, K., Oura, H., Nonaka, G. and Nishioka, I.: Effects of rhubarb tannins on uremic toxins. *Nephron* **58**, 155-160, 1991.
53) Yokozawa, T., Zheng, P.D., Oura, H., Hattori, M., Kuwayama, N. and Takaku, A.: Ompi-to relieves acidosis in rats given adenine. *J. Med. Pharm. Soc. WAKAN-YAKU* **9**, 55-58, 1992.
54) Yokozawa, T., Maeda, K., Mitsuma, T., Toriizuka, K. and Oura, H.: Effects of Ompi-to on renal anemia and platelet aggregation activity. *J. Med. Pharm. Soc. WAKAN-YAKU* **9**, 137-142, 1992.
55) Yokozawa, T., Fujioka, K., Oura, H., Nonaka, G. and Nishioka, I.: Effects of rhubarb tannins on renal function in rats with renal failure. *Jpn. J. Nephrol.* **35**, 13-18, 1993.
56) Yokozawa, T., Oura, H., Iwano, M., Dohi, K. and Hattori, M.: Inhibitory effects of crude drug components on the proliferation of human mesangial cells. *Jpn. J. Nephrol.* **35**, 321-327, 1993.
57) Yokozawa, T., Oura, H., Hattori, M., Iwano, M. and Dohi, K.: Effects of Wen-Pi-Tang and its crude drug extracts on proliferation of cultured mouse mesangial cells. *Phytother. Res.* **8**, 170-173, 1994.
58) Yokozawa, T., Dong, E., Liu, Z.W. and Oura, H.: Antiperoxidation activity of Wen-Pi-Tang *in vitro*. *Nat. Med.* **50**, 243-246, 1996.
59) Yokozawa, T., Dong, E., Oura, H., Nishioka, I., Kawai, Y. and Gemba, M.: Protective effects of Wen-Pi-Tang against cultured renal epithelial cellular injury. *Phytomedicine* **4**, 245-250, 1997.
60) Yokozawa, T., Dong, E., Yasui, T. and Muraguchi, A.: Protective effect of Wen-Pi-Tang against apoptosis of cultured renal epithelial cells. *Phytother. Res.* **12**, 135-137, 1998.
61) Yokozawa, T., Chen, C.P., Tanaka, T. and Kouno, I.: Isolation from Wen-Pi-Tang of the active principles possessing antioxidation and radical-scavenging activities. *Phytomedicine* **5**, 367-373, 1998.
62) Yokozawa, T., Sekiya, M., Rhyu, D.Y., Hattori, M. and Chung, H.Y.: Radical-scavenging activity of Wen-Pi-Tang and its component crude drugs: with special reference to the effects on nitric oxide, superoxide and peroxynitrite. *J. Trad. Med.* **17**, 41-47, 2000.
63) Yokozawa, T., Dong, E. and Chen, C.P.: Protection of the kidney by Wen-Pi-Tang against ischemia-reperfusion injury. *Phytomedicine* **7**, 185-189, 2000.
64) Cho, E.J., Yokozawa, T., Rhyu, D.Y., Mitsuma, T., Terasawa, K. and Park, J.C.: Protective activity from hydrophilic and lipophilic free radical generators of Wen-Pi-Tang and its crude drug extracts in LLC-PK$_1$ cells. *J. Trad. Med.* **17**, 245-252, 2000.
65) Yokozawa, T., Rhyu, D.Y. and Owada, S.: Increase of radical in rats with adenine-induced renal failure is suppressed by Wen-Pi-Tang. *J. Trad. Med.* **18**, 147-153, 2001.
66) Gobe, G., Willgoss, D., Hogg, N., Schoch, E. and Endre, Z.: Cell survival or death in renal tubular epithelium after ischemia-reperfusion injury. *Kidney Int.* **56**, 1299-1304, 1999.
67) Schena, F.P., Grandaliano, G. and Gesualdo, L.: The role of tubular cells in the progression of renal damage: guilty or innocent? *Ren. Fail.* **23**, 589-596, 2001.
68) Rhyu, D.Y., Yokozawa, T., Cho, E.J. and Park, J.C.: Prevention of peroxynitrite-induced renal injury through modulation of peroxynitrite production by the Chinese prescription Wen-Pi-Tang. *Free Radic. Res.* **36**, 1261-1269, 2002.
69) Sokolovsky, M., Riordan, J.F. and Vallee, B.L.: Conversion of 3-nitrotyrosine to 3-aminotyrosine in peptides and proteins. *Biochem. Biophys. Res. Commun.* **27**, 20-25, 1967.
70) Fukuyama, N., Takebayashi, Y., Hida, M., Ishida, H., Ichimori, K. and Nakazawa, H.: Clinical evidence of peroxynitrite formation in chronic renal failure patients with septic shock. *Free Radic. Biol. Med.* **22**, 771-774, 1997.
71) Kaur, H. and Halliwell, B.: Evidence for nitric oxide mediated oxidative damage in chronic inflammation. Nitrotyrosine in serum and synovial fluid from rheumatoid patients. *FEBS Lett.* **350**, 9-12, 1994.
72) ter Steege, J.C.A., Koster-Kamphuis, L., van Straaten, E.A., Forget, P.P. and Buurman, W.A.: Nitrotyrosine in plasma of celiac disease patients as detected by a new sandwich ELISA. *Free Radic. Biol. Med.* **25**, 953-963, 1998.
73) Noiri, E., Nakao, A., Uchida, K., Tsukahara, H., Ohno, M., Fujita, T., Brodsky, S. and Goligorsky, M.S.: Oxidative and nitrosative stress in acute renal ischemia. *Am. J. Physiol.* **281**, F948-F957, 2001.
74) Xia, Y. and Zweier, J.L.: Substrate control of free radical generation from xanthine oxidase in the postischemic heart. *J. Biol.*

Chem. **270**, 18797-18803, 1995.

75) Houston, M., Chumley, C., Radi, R., Rubbo, H. and Freeman, B.A.: Xanthine oxidase reaction with nitric oxide and peroxynitrite. *Arch. Biochem. Biophys.* **355**, 1-8, 1998.

76) Lee, C.I., Liu, X. and Zweier, J.L.: Regulation of xanthine oxidase by nitric oxide and peroxynitrite. *J. Biol. Chem.* **275**, 9369-9376, 2000.

77) Brown, G.C.: Reversible binding and inhibition of catalase by nitric oxide. *Eur. J. Biochem.* **232**, 188-191, 1995.

78) Asahi, M., Fujii, J., Suzuki, K., Seo, H.G., Kuzuya, T., Hori, M., Tada, M., Fujii, S. and Taniguchi, N.: Inactivation of glutathione peroxidase by nitric oxide. Implication for cytotoxicity. *J. Biol. Chem.* **270**, 21035-21039, 1995.

79) Yokozawa, T., Rhyu, D.Y., Cho, E.J. and Aoyagi, K.: Protective activity of (-)-epicatechin 3-*O*-gallate against peroxynitrite-mediated renal damage. *Free Radic. Res.* **37**, 561-572, 2003.

80) Lin, K.T., Xue, J.Y., Nomen, M., Spur, B. and Wong, P.Y.: Peroxynitrite-induced apoptosis in HL-60 cells. *J. Biol. Chem.* **270**, 16487-16490, 1995.

81) Lieberthal, W. and Levine, J.S.: Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. *Am. J. Physiol.* **271**, F477-F488, 1996.

82) Sandoval, M., Zhang, X.J., Liu, X., Mannick, E.E., Clark, D.A. and Miller, M.J.: Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: attenuation by L-ascorbic acid. *Free Radic. Biol. Med.* **22**, 489-495, 1997.

83) Doulias, P.T., Barbouti, A., Galaris, D. and Ischiropoulos, H.: SIN-1-induced DNA damage in isolated human peripheral blood lymphocytes as assessed by single cell gel electrophoresis (comet assay). *Free Radic. Biol. Med.* **30**, 679-685, 2001.

84) Poot, M., Schindler, D., Kubbies, M., Hoehn, H. and Rabinovitch, P.S.: Bromodeoxyuridine amplifies the inhibitory effect of oxygen on cell proliferation. *Cytometry* **9**, 332-338, 1988.

85) Poot, M.: Oxidants and antioxidants in proliferative senescence. *Mutat. Res.* **256**, 177-189, 1991.

86) Hodgson, J.M., Puddey, I.B., Burke, V., Beilin, L.J. and Jordan, N.: Effects on blood pressure of drinking green and black tea. *J. Hypertens.* **17**, 457-463, 1999.

87) Yang, C.S. and Wang, Z.Y.: Tea and cancer. *J. Natl. Cancer Inst.* **85**, 1038-1049, 1993.

88) Dreosti, I.E.: Bioactive ingredients: antioxidants and polyphenols in tea. *Nutr. Rev.* **54**, s51-s58, 1996.

89) Yokozawa, T., Oura, H., Sakanaka, S., Ishigaki, S. and Kim, M.: Depressor effect of tannin in green tea on rats with renal hypertension. *Biosci. Biotechnol. Biochem.* **58**, 855-858, 1994.

90) Nakagawa, T., Yokozawa, T., Terasawa, K., Shu, S. and Juneja, L.R.: Protective activity of green tea against free radical- and glucose-mediated protein damage. *J. Agric. Food Chem.* **50**, 2418-2422, 2002.

91) Davies, K.J. and Goldberg, A.L.: Proteins damaged by oxygen radicals are rapidly degraded in extracts of red blood cells. *J. Biol. Chem.* **262**, 8227-8234, 1987.

92) Meucci, E., Mordente, A. and Martorana, G.E.: Metal-catalyzed oxidation of human serum albumin: conformational and functional changes. Implications in protein aging. *J. Biol. Chem.* **266**, 4692-4699, 1991.

93) Monnier, V.M. and Cerami, A.: Nonenzymatic browning *in vivo*: possible process for aging of long-lived proteins. *Science* **221**, 491-493, 1981.

94) Smith, M.A., Taneda, S., Richey, P.L., Miyata, S., Yan, S.D., Stern, D., Sayre, L.M., Monnier, V.M. and Perry, G.: Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc. Natl. Acad. Sci. USA* **91**, 5710-5714, 1994.

95) Vlassara, H.: Recent progress in advanced glycation end products and diabetic complications. *Diabetes* **46**, s19-s25, 1997.

96) Fu, M.X., Wells-Knecht, K.J., Blackledge, J.A., Lyons, T.J., Thorpe, S.R. and Baynes, J.W.: Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics, mechanisms, and inhibition of late stages of the Maillard reaction. *Diabetes* **43**, 676-683, 1994.

97) Yaylayan, V.A. and Huyghues-Despointes, A.: Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **34**, 321-369, 1994.

98) Yokozawa, T., Cho, E.J., Hara, Y. and Kitani, K.: Antioxidative activity of green tea treated with radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride. *J. Agric. Food Chem.* **48**, 5068-5073, 2000.

99) Matsura, T., Yamada, K. and Kawasaki, T.: Difference in antioxidant activity between reduced coenzyme Q_9 and reduced coenzyme Q_{10} in the cell: studies with isolated rat and guinea pig hepatocytes treated with a water-soluble radical initiator. *Biochim. Biophys. Acta* **1123**, 309-315, 1992.

100) Martin, A., Wu, D.Y., Baur, W., Meydani, S.N., Blumberg, J.B. and Meydani, M.: Effect of vitamin E on human aortic endothelial cell responses to oxidative injury. *Free Radic. Biol. Med.* **21**, 505-511, 1996.

101) Rapin, J.R., Zaibi, M. and Drieu, K.: *In vitro* and *in vivo* effects of an extract of Ginkgo biloba (EGb 761), ginkgolide B, and bilobalide on apoptosis in primary cultures of rat hippocampal neurons. *Drug Dev. Res.* **45**, 23-29, 1998.

102) Terao, E. and Niki, E.: Damage to biological tissues induced by radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride and its inhibition by chain-breaking antioxidants. *Free Radic. Biol. Med.* **2**, 193-201, 1986.

103) Yokozawa, T., Chung, H.Y., He, L.Q. and Oura, H.: Effectiveness of green tea tannin on rats with chronic renal failure. *Biosci. Biotech. Biochem.* **60**, 1000-1005, 1996.

104) Schrier, R.W., Harris, D.C.H., Chan, L., Shapiro, J.I. and Caramelo, C.: Tubular hypermetabolism as a factor in the progression of chronic renal failure. *Am. J. Kidney Dis.* **12**, 243-249, 1988.

105) Harris, D.C.H., Chan, L. and Schrier, R.W.: Remnant kidney hypermetabolism and progression of chronic renal failure. *Am. J. Physiol.* **254**, F267-F276, 1988.

106) Yokozawa, T. and Dong, E.: Free radicals in food. Chemistry, nutrition, and health effects. Radical-scavenging activity of green tea polyphenols. ACS Symposium Series, Am. Chem. Soc. Washington, DC., pp.224-240, 2002.

107) Yokozawa, T., Fujitsuka, N. and Oura, H.: Production of methylguanidine from creatinine in normal rats and rats with renal failure. *Nephron* **56**, 249-254, 1990.

108) Yokozawa, T., Fujitsuka, N. and Oura, H.: Studies on the precursor of methylguanidine in rats with renal failure. *Nephron* **58**, 90-94, 1991.

109) Ienaga, K., Nakamura, K., Yamakawa, M., Toyomaki, Y., Matsuura, H., Yokozawa, T., Oura, H. and Nakano, T.: The use of ^{13}C-labelling to prove that creatinine is oxidized by mammals into creatol and 5-hydroxy-1-methylhydantoin. *J. Chem. Soc., Chem. Commun.* 509-510, 1991.

110) Nakamura, K., Ienaga, K., Yokozawa, T., Fujitsuka, N. and Oura, H.: Production of methylguanidine from creatinine *via* creatol by active oxygen species. Analyses of the catabolism *in vitro*. *Nephron* **58**, 42-46, 1991.

111) Yokozawa, T., Fujitsuka, N., Oura, H., Mori, A. and Kashiwagi, H.: Determination of radical species in the kidney of rats with chronic renal failure by the spin trapping method. *Nephron* **70**, 382-384, 1995.

112) Yokozawa, T., Fujitsuka, N., Oura, H., Ienaga, K. and Nakamura, K.: *In vivo* effect of hydroxyl radical scavenger on methylguanidine production from creatinine. *Nephron* **75**, 103-105, 1997.

113) Terao, J., Piskula, M. and Yao, Q.: Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipids bilayers. *Arch. Biochem. Biophys.* **308**, 278-284, 1994.

114) Yokozawa, T., Dong, E., Chung, H.Y., Oura, H. and Nakagawa, H.: Inhibitory effect of green tea on injury to a cultured renal epithelial cell line, LLC-PK_1. *Biosci. Biotech. Biochem.* **61**, 204-206, 1997.

115) Yokozawa, T., Dong, E. and Oura, H.: Proof that green tea tannin suppresses the increase in the blood methylguanidine level associated with renal failure. *Exp. Toxic. Pathol.* **49**, 117-122, 1997.

116) Yokozawa, T., Dong, E., Nakagawa, T., Kashiwagi, H., Nakagawa, H., Takeuchi, S. and Chung, H.Y.: *In vitro* and *in vivo* studies on the radical-scavenging activity of tea. *J. Agric. Food Chem.* **48**, 2143-2150, 1998.

117) Chung, H.Y., Yokozawa, T., Soung, D.Y., Kye, I.S., No, J.K. and Baek, B.S.: Peroxynitrite-scavenging activity of green tea tannin. *J. Agric. Food Chem.* **46**, 4484-4486, 1998.

118) Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. and Rice-Evans, C.: Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **322**, 339-346, 1995.

119) Zhang, A., Zhu, Q.Y., Luk, Y.S., Ho, K.Y., Fung, K.P. and Chen, Z.Y.: Inhibitory effects of jasmine green tea epicatechin isomers on free radical-induced lysis of red blood cells. *Life Sci.* **61**, 383-394, 1997.