Chronic green tea extract supplementation reduces hemodialysis-enhanced production of hydrogen peroxide and hypochlorous acid, atherosclerotic factors, and proinflammatory cytokines1–3

Shih-Ping Hsu, Ming-Shiou Wu, Chih-Ching Yang, Kuo-Chin Huang, Shaw-Yih Liou, Su-Ming Hsu, and Chiang-Ting Chien

ABSTRACT

Background: Oxidative stress increases in patients with end-stage renal disease and exaggerates the related comorbidities.

Objective: The aim of the study was to evaluate the effects of supplementation with decaffeinated green tea extract (catechins) on hemodialysis-induced reactive oxygen species, atherosclerotic disease risk factors, and proinflammatory cytokines.

Design: We enrolled 6 healthy subjects and 54 hemodialysis patients for the study. First, the pharmacokinetics of one oral dose of catechins was compared between healthy subjects (n = 6) and hemodialysis patients (n = 10). Second, in the 10 hemodialysis patients, we compared the antioxidant effects of 3 different doses (0, 455, and 910 mg) of oral catechins with that of oral vitamin C (500 mg) during a hemodialysis session. Third, the other 44 hemodialysis patients participated in a 7-mo interventional study, in which 30 patients received placebo throughout and 14 patients received catechins (455 mg/d) from the third to the fifth month.

Results: After one oral dose, the hemodialysis patients (n = 10) had later peaks and slower decay of plasma catechins than did the healthy subjects. In the 10 hemodialysis patients, catechin supplementation reduced hemodialysis-enhanced plasma hypochlorous acid activity more effectively than did placebo or vitamin C. Between treatments with 455 or 910 mg catechins, no significant difference was found in the reduction of plasma hypochlorous acid activity. Catechins also significantly reduced proinflammatory cytokine expression enhanced by hemodialysis. In the 7-mo interventional study, the 14 patients who received daily supplementation of catechins for 3 mo had less predialysis plasma hydrogen peroxide activity, lower hypochlorous acid activity, and lower phosphatidylcholine hydroperoxide, C-reactive protein, and proinflammatory cytokine concentrations than did the 30 hemodialysis patients who received placebo.


KEY WORDS Hemodialysis, oxidative stress, catechins, proinflammation, antinflammation

INTRODUCTION

A cytokine-driven acute-phase inflammatory response and an increase in oxidative stress status are closely associated with the pathogenesis of cardiovascular disease (1). In end-stage renal disease (ESRD) patients undergoing hemodialysis, the interaction of blood with nonbiological materials of the extracorporeal circuit may activate polymorphonuclear leukocytes (PMNs) to increase the production of reactive oxygen species (ROS; 2–4), which impairs neighboring tissues and cells and evokes an inflammatory response (5). Two major ROS generated from activated PMNs are hydrogen peroxide and hypochlorous acid (4, 5), which potentially oxidize LDL. Oxidized LDL (oxLDL) increases the adhesion of monocytes to the endothelium, the transmigration of macrophages into foam cells, and the impairment of endothelium-dependent vasorelaxation (6–8). The concentration of phosphatidylincholine hydroperoxide, a primary lipid peroxidation product of LDL and VLDL, is positively correlated with the amount of hydrogen peroxide and hypochlorous acid (4). Greater phosphatidylincholine hydroperoxide concentrations contribute to aging and cardiovascular disease (4, 5). Hydrogen peroxide and hypochlorous acid in greater concentrations can also serve as second messengers in the induction of proinflammatory nuclear transcription factor-κB (NF-κB)–regulated genes (9), which include soluble intercellular adhesion molecule 1 (sICAM-1), monocyte chemoattractant protein 1 (MCP-1), and...
tumor necrosis factor-α (TNF-α) (10). These cytokines are important mediators of inflammation. The plasma concentrations of proinflammatory cytokines were significantly higher in patients with renal failure than in healthy subjects (11). Expression of these proinflammatory cytokines can be further augmented by the dialysis process (12). Therefore, greater oxidative stress and higher concentrations of proinflammatory cytokines may be important targets in nutritional and pharmacologic therapies for uremic patients undergoing hemodialysis.

Recent findings show that antioxidant supplementation inhibits the progression of atherosclerosis and inflammation. However, most of the antioxidants tested to date, eg, probucol, butylated hydroxytoluene, and \( N,N' \)-diphenylphenylenediamine, carry potential side effects that preclude their utility in human clinical trials (13). Green tea extracts containing (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate (EGCG), and (-)-epigallocatechin gallate (EGC) are considered to exert protective effects against cancer (14) and inflammatory (15) and cardiovascular (16) diseases. Catechins can inhibit proinflammatory and proapoptotic oxidative injury via a reduction in the production of ROS, the translocation of NF-κB and activated protein 1, and the expression of ICAM-1 (17, 18). Furthermore, catechins are more effective than are vitamins C and E at scavenging plasma hydrogen peroxide and hypochlorous acid activities (4). We therefore speculated that supplementation with chronic green tea extract may provide an alternative approach to reducing chronic hemodialysis-enhanced ROS production, atherosclerotic risk factors, and proinflammatory cytokines in ESRD patients.

SUBJECTS AND METHODS

Subjects

We enrolled 54 patients (37 men, 17 women) in the study. Excluded were subjects who, in the preceding 3 mo, had habitually smoked; had had a malignancy, inflammatory disorder, or chronic or acute infection; had used vitamin C or vitamin E supplements; had received oral or intravenous iron therapy; or had been treated with antiinflammatory drugs. Before the study, the subjects had been undergoing maintenance hemodialysis at Taipei City United Hospital, He-Ping Branch, for >3 mo. Hemodialysis continued and was performed on a polysulfone dialyzer (APS-1050; Asahi Medical Co Ltd, Tokyo, Japan). All of the patients received a standardized hemodialysis prescription as follows: bicarbonate dialysate flow: 500 mL/min; blood flow: 250–300 mL/min; 4 h/session; 3 sessions/wk. The clearance [calculated as \( Kt/V \), where \( K \) is a dialyzer clearance, \( t \) is hemodialysis time, and \( V \) is the volume of distribution of urea (approximately equal to total body weight)] in all patients was maintained between 1.2 and 1.5.

In the first part of the study, we evaluated the plasma catechin concentrations in 6 healthy subjects and 10 ESRD patients at serial time points after the administration of a single oral dose (455 mg) of decaffeinated green tea extracts (catechins tablet; Numen Biotech, Taipei, Taiwan), which consisted of various types of catechins (2.29 mg catechins, 7.98 mg epicatechin, 9.53 mg ECG, 10.66 mg gallocatechin, 13.79 mg epigallocatechin, and 47.31 mg EGC per tablet). Because one cup of green tea contains ~100–150 mg catechins, the total amount of catechins (455 mg) ingested was comparable to 4 cups of green tea (19). In the second part of the study, we compared the antioxidant effect of catechins (455 mg) with that of oral vitamin C (500 mg) in the 10 ESRD patients. Moreover, we compared the effects of 3 different doses (0, 455, and 910 mg) of catechins on plasma catechin concentrations, ROS, myeloperoxidase activity, and di-tyrosine and phosphatidylcholine hydroperoxide concentrations during a single hemodialysis session. The higher dose of catechins (910 mg) was approximately equivalent to that from 8 cups of green tea.

In the third part of the study, we measured the long-term outcome effects of catechin supplementation in ESRD patients. The phosphatidylcholine hydroperoxide concentration, a primary lipid peroxidation product of LDL or VLDL, is positively correlated with the amount of hydrogen peroxide and hypochlorous acid (4) and contributes to aging and cardiovascular disease (4, 5). On the basis of an \( \alpha \) level of 0.05 and power >90% from our preliminary data of phosphatidylcholine hydroperoxide concentrations in the short-term experiment (the second part of the experiment) and in the 1-mo experiment, there was a significant effect between 10 experimental patients and 10 control subjects. However, considering the drop-out rate, other markers, statistical power, and economic efficiency, we finally recruited 14 experimental patients and 30 control subjects in the clinical trial. Despite the unequal sample sizes, satisfactory statistical power could still be achieved by adopting a ratio of 1:2 (experimental patients:control subjects), with substantial cost savings for economic efficiency (20).

The baseline characteristics of the patients in both groups are shown in Table 1. During the 7-mo study period, the subjects did not take drugs with established or potential oxidizing effect, nor did they take antioxidants such as vitamin C or vitamin E. During the first 2 observational months, all 44 patients received placebo. In the following 3 mo, the 14 patients in the catechins group received 455 mg catechins once a day. In the last 2 observational months, the 14 patients received only placebo and no longer received catechins.

All subjects provided written informed consent. The clinical trial followed the Declaration of Helsinki and was approved by the Human Research Committee of the National Taiwan University Hospital.

Analysis of catechins in plasma by using high-performance liquid chromatography

The standard samples of catechins, epicatechin, galloatechin, epigallocatechin, ECG, and EGCG were purchased from Sigma Chemical Co (St Louis, MO). Oxalic acid, ethanol, and monosodium phosphate were purchased from Merck (Rahway, NJ). Gradient mobile phase A and B solutions were obtained from ESA Inc (Bedford, MA). A standard stock mixture of catechins, epicatechin, galloatechin, epigallocatechin, ECG, and EGCG at 100 \( \mu \)g/mL was prepared in 10 mmol/L oxalic acid solution and stored in small aliquots at ~80 °C until used. The stock solutions remained stable for >6 mo.

The distribution of catechins in plasma and standards was determined by a gradient HPLC-coulometric electrode array system (ESA Inc). One milliliter of the samples was subjected to aluminum oxide solid-phase extraction (ESA Inc), and the resulting samples in 1 mol NaH\(_2\)PO\(_4\)/L (pH 2.5) were applied to the HPLC with the use of an autosampler (model 542; ESA Inc) and an electrochemical detector (model 5600A; ESA Inc). The separating conditions were as follows: column, HR-80 (C-18, 3 \( \mu \)m,
Measurement of myeloperoxidase activity and lipid and peroxidation products

The 2 major ROS generated from activated PMNs via the myeloperoxidase system are hydrogen peroxide and hypochlorous acid, which can produce the primary lipid peroxidation product phosphatidylcholine hydroperoxide and the protein oxidation product dityrosine (4, 5). Oxidized phosphatidylcholine, one of the key molecules in oxLDL, is capable of inducing monocyte adhesion to endothelial cells and neutrophil migration and is directly involved in the early development of atherosclerosis (24). Measurement of oxLDL in circulating plasma by using phosphatidylcholine hydroperoxide could provide the means to monitor the behavior of oxidized phosphatidylcholine particles as part of oxLDL in plasma (4, 5). Additionally, previous studies showed that 254 mg green tea extracts reduced plasma phosphatidylcholine hydroperoxide concentrations from 73.7 ± 32.4 to 44.6 ± 8.9 pmol/mL, whereas 600 mg green tea extracts reduced urinary isoprostane formation from 74 ± 4 to 63 ± 4 pg/mL (19, 25). The amounts of phosphatidylcholine hydroperoxide in plasma were determined in duplicate by chemiluminescence-emitting substance [luminol (5-amino-2,3-dihydro-1,4-phthalazinedione); Sigma] with the use of a multiwavelength chemiluminescence spectrum analyzer (CLASP2; Tohoku Electronic Industrial Co, Sendai, Japan) as described previously (4, 5). The chemiluminescence emitted from the plasma samples was assigned as “reference hydrogen peroxide counts” (RH2O2) or “reference hypochlorous acid counts” (ROHCl). Higher RH2O2 and ROHCl indicate lower antioxidant activity, higher ROS activity, or both in the tested plasma.

Blood samples and biochemical analysis

Ten milliliters of blood was drawn via the arterial line of the hemodialysis circuit, just before the start and the end of a hemodialysis session, respectively. Blood was then collected into a heparinized test tube and processed within 1 h. Plasma was separated from blood cells by centrifugation at 750 × g for 5 min at 4 °C. After separation, the plasma was immediately stored at −70 °C and was analyzed within 2 wk.

Serum paraoxonase 1 assay

Serum paraoxonase 1 is an oxidant-sensitive enzyme associated with HDL that inhibits the atherogenic oxidation of LDL (21). High concentrations of hypochlorous acid, but not of hydrogen peroxide, can reduce paraoxonase 1 activity in hemodialysis patients (22). An aliquot of the plasma reaction mixture was diluted 1:40 in 20 mmol/L tris buffer (pH 7.4) and was assayed for paraoxonase 1 activity as described previously (21).

Measurement of specific plasma reactive oxygen species activity

Our previous study showed that hydrogen peroxide and hypochlorous acid counts were negatively correlated with the total antioxidant status and were positively correlated with phosphatidylcholine hydroperoxide (primary lipid peroxidation; 5). Additionally, in a nonlinear functional analysis, the hydrogen peroxide counts of all plasma samples were positively correlated with hemolysis scores (23). This correlation indicates that measurements of the hydrogen peroxide and hypochlorous acid concentrations reflect the degrees of lipid and protein peroxidation. We measured specific chemiluminescence signals of hydrogen peroxide and hypochlorous acid emitted from the plasma amplified by chemiluminescence-emitting substance [luminol (5-amino-2,3-dihydro-1,4-phthalazinedione); Sigma] with the use of a multiwavelength chemiluminescence spectrum analyzer (CLASP2; Tohoku Electronic Industrial Co, Sendai, Japan) as described previously (4, 5). The chemiluminescence emitted from the plasma samples was assigned as “reference hydrogen peroxide counts” (RH2O2) or “reference hypochlorous acid counts” (ROHCl). Higher RH2O2 and ROHCl indicate lower antioxidant activity, higher ROS activity, or both in the tested plasma.

TABLE 1
Comparisons in the baseline characteristics of the patients (n = 44) enrolled for evaluation of the long-term effects of dietary catechins

<table>
<thead>
<tr>
<th>Variable</th>
<th>Catechins group (n = 14)</th>
<th>Control group (n = 30)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>60.1 ± 14.8 (19.2–87.0)²</td>
<td>58.1 ± 15.0 (25.7–85.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/4</td>
<td>22/8</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of HD (mo)</td>
<td>39.7 ± 34.5 (6.4–171.9)</td>
<td>42.0 ± 35.8 (7.4–160.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus (yes/no)</td>
<td>7/7</td>
<td>13/17</td>
<td>NS</td>
</tr>
<tr>
<td>Kt/V¹</td>
<td>1.36 ± 0.10²</td>
<td>1.32 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.9 ± 0.5</td>
<td>4.0 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>6.8 ± 0.8</td>
<td>6.1 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ HD, hemodialysis.
² Independent t test or chi-square method.
³ ± SD; range in parentheses (all such values).
⁴ Calculation of urea clearance (Daugirdas method), where K is dialyzer clearance, t is hemodialysis time, and V is the volume of distribution of urea (approximately equal to total body weight).
⁵ ± SD (all such values).

4.6 mm × 80 mm; ESA Inc); column temperature, 25 °C gradient mobile phase A (containing phosphate buffer and an ion-pairing agent, 45–0171; ESA Inc) and gradient mobile phase B (containing methanol, phosphate buffer, and an ion-pairing agent; ESA Inc); 1 mL/min flow rate. The optional channel potential was set to 220 mV. The respective plasma catechin concentration was calculated from the area under the curve and was compared with an external standard area under the curve of catechins, epicatechin, gallocatechin, epigallocatechin, ECG, and EGCG.
measured in plasma by use of an enzyme-linked immunosorbent assay kit (Calbiochem, San Diego, CA).

C-reactive protein, proinflammatory cytokines, and multiple cytokine antibody array

C-reactive protein (CRP), a marker of the acute-phase response and chronic inflammation, is involved in the pathogenesis of atherosclerosis (26). Serum CRP concentrations were measured by using an immunometric kit (717-80A3; Iatron Laboratories Inc, Tokyo, Japan). The reader (autoanalyzer) was Tectron U-240 Plus (Iatron Laboratories Inc). Quantification of sICAM-1, MCP-1, and TNF-α was determined by use of enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, MN). Multiple cytokine expression concentrations were simultaneously determined by using the RayBio human cytokine protein array VI and VII (RayBiotech Inc, Norcross, GA) according to the manufacturer’s instructions.

Statistical analyses

All values are expressed as means ± SEMs unless stated otherwise. Between-group comparisons were performed by using unpaired t tests or analysis of variance with Bonferroni method as post hoc analysis; within-group comparisons were performed by using paired t tests or repeated-measures analysis of variance with Bonferroni method as post hoc analysis. A value of P < 0.05 indicated statistical significance. All computations were performed with SPSS for WINDOWS software (version 13.0; SPSS Inc, Chicago, IL).

RESULTS

Plasma concentrations of catechins

We assayed the plasma catechin concentrations in healthy control subjects (n = 6) and ESRD patients (n = 10) for 24 h after a single oral dose of catechins (455 mg; Figure 1). Although 6 typical components of catechins appeared in the standard assay, after 30–60 min of oral catechin administration, only 4 catechins were detected in the plasma of the 6 healthy control subjects. These 4 catechins decayed after ≈4 h. In ESRD patients (n = 10), after 30–60 min of catechin ingestion, these catechins were also detected in the plasma. These 4 catechin concentrations, however, decayed after ≈6 h. When the areas under the curve of the 4 catechins were compared, there were no significant differences between the 2 groups.

Catechins reduced hemodialysis-enhanced reference hydrogen peroxide counts and reference hypochlorous acid counts and restored paraoxonase 1 activity

A typical chemiluminescence emission from RH₂O₂ and RHOCI in the pre- and posthemodialysis plasma samples from one ESRD patient who received placebo, vitamin C, or catechins is shown in Figure 2A. The chemiluminescence counts read at maximum emission (460 nm) were 52.2 ± 17.1 and 138.5 ± 62.9 for plasma epigallocatechin (EGC) concentrations, 52.6 ± 29.1 and 97.9 ± 53.1 for epigallocatechin gallate (EGCG), 13.1 ± 4.9 and 44.4 ± 24.3 for epicatechin gallate (ECG), and 13.1 ± 4.9 and 22.3 ± 4.5 for epicatechin (EC). None of the differences in AUCs were significant.

FIGURE 1. Mean (±SEM) plasma concentrations of catechins after a single oral dose of green tea extracts (455 mg catechins) in 6 healthy control subjects and 10 end-stage renal disease (ESRD) patients. In the healthy group and the ESRD group, the respective areas under the curve (AUCs; mean h x ng/mL) were 52.2 ± 17.1 and 138.5 ± 62.9 for plasma epigallocatechin (EGC) concentrations, 52.6 ± 29.1 and 97.9 ± 53.1 for epigallocatechin gallate (EGCG), 13.1 ± 4.9 and 44.4 ± 24.3 for epicatechin gallate (ECG), and 13.1 ± 4.9 and 22.3 ± 4.5 for epicatechin (EC). None of the differences in AUCs were significant.

Effects of catechins on reducing hemodialysis-enhanced reactive oxygen species, myeloperoxidase activity, and phosphatidylcholine hydroperoxide and dityrosine concentrations

Hemodialysis enhanced plasma RH₂O₂, RHOCI, plasma myeloperoxidase activity, and phosphatidylcholine hydroperoxide and dityrosine concentrations in the 10 ESRD patients receiving placebo (Figure 3). When the same patients received either 455 or 910 mg catechins, the hemodialysis-enhanced plasma RHOCI was significantly reduced, but plasma RH₂O₂, myeloperoxidase activity, and concentrations of phosphatidylcholine hydroperoxide and dityrosine were not. Except for epigallocatechin, there were no significant differences between the area under the curve of 455 mg catechins and that of 910 mg catechins.
Catechins reduced hemodialysis-enhanced proinflammatory markers

We also analyzed multiple cytokines in the posthemodialysis plasma of the 10 ESRD patients after the administration of placebo or catechins. In the posthemodialysis plasma, a single dose (455 mg) of catechins down-regulated one soluble apoptosis mediator (Fas/TNF receptor superfamily, member 6, Fas ligand), 3 proinflammatory mediators [soluble circulating receptor of interleukin-6 (IL-6R), interleukin-8 (IL-8), and neutrophil-activating protein 2 (NAP-2)], and 3 antiinflammatory mediators [IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors (sTNF-RI and sTNF-RID)], whereas none of these mediators were down-regulated in the posthemodialysis plasma in the subjects receiving placebo (Figure 4).

Long-term effects of catechins: a 7-mo study

In the longitudinal study, we recorded monthly the prehemodialysis plasma concentrations of hydrogen peroxide, hypochlorous acid, phosphatidylcholine hydroperoxide, CRP, sICAM-1, MCP-1, and TNF-α in 44 ESRD patients: 14 patients in the catechins group and 30 in the control group. There was no significant difference between the 2 groups of patients in the clinical
characteristics and baseline blood chemistry (Table 1). As shown in Figure 5, during the first 2 observational months, there was no significant difference between the 2 groups with regard to the prehemodialysis plasma concentrations of RH$_2$O$_2$, RHOCl, phosphatidylcholine hydroperoxide, CRP, sICAM-1, MCP-1, and TNF-$\alpha$. In the following 3 interventional months, the above 7 variables in the catechins group decreased and were significantly lower than in the control group by 5 mo. In the last 2 study months, during which catechin supplementation was discontinued, the above 7 variables in the catechins group returned to baseline and were no longer significantly different from those in the control group.

**DISCUSSION**

In the present study, we showed that the plasma values of epicatechin, ECG, epigallocatechin, and EGCG in the healthy control subjects peaked at 30 min and decayed at 4 h after the ingestion of catechins. However, in ESRD patients, the concentrations of these 4 catechins peaked at 1 h and decayed at 6 h. Hemodialysis evoked a large amount of ROS as plasma RH$_2$O$_2$ and RHOCl activities, which in turn increased phosphatidylcholine hydroperoxide concentrations. In comparison with vitamin C, catechin supplementation was more effective in reducing hemodialysis-enhanced plasma RHOCl activities and restoring paraoxonase 1 activity. Furthermore, the postdialysis plasma concentrations of proapoptosis mediators, proinflammatory cytokines, and leukocyte-mediated antiinflammatory molecules were down-regulated significantly by catechins. During the 3 experimental months, the predialysis plasma concentrations of phosphatidylcholine hydroperoxide, TNF-$\alpha$, sICAM-1, MCP-1, and CRP in the patients receiving catechins daily were lower than both their own baseline values and those in the control group.

The increased ROS found in patients with ESRD undergoing chronic hemodialysis could originate from complement-,
platelet-, and even dialysis membrane–activated leukocytes (2–4). A recent study by our group showed that a single session of hemodialysis activates blood to produce a 14-fold increase in ROS ($71\%$ H$_2$O$_2$, $15\%$ O$_2^\cdot$, and $14\%$ HOCl; 23). Vitamin C efficiently palliates augmented superoxide anion and hydrogen peroxide (86% of total ROS), but not hypochlorous acid (23). The present study and our recent reports clearly indicate that catechins scavenge superoxide anion (17), hydrogen peroxide, and hypochlorous acid more efficiently than does vitamin C (4). In hemodialysis patients, overproduction of hypochlorous acid contributes to low serum paraoxonase 1 activity, which may promote the atherogenic oxidation of LDL (21). Increased hypochlorous acid amplifies the hydrogen peroxide–induced vascular injury by additional impairment of endothelium-dependent relaxation (27). Therefore, catechins exert a more efficient protection than does vitamin C against hemodialysis-induced atherosclerotic danger.

Despite efficient clearance of blood urea nitrogen and creatinine, hemodialysis does not mechanically remove oxidized products, such as oxLDL or phosphatidylcholine hydroperoxide, from ESRD patients (4). Moreover, hemodialysis treatment enhances the accumulation of phosphatidylcholine hydroperoxide (4). The initial stage of atherosclerotic plaque formation involves oxidation of the phosphatidylcholine moiety of LDL and subsequent uptake by macrophages (27). Hemodialysis intensifies lipid peroxidation, and such accumulation of oxLDL and phosphatidylcholine hydroperoxide could induce vasocostriction in microvascular beds (28) and account for the accelerated progression of atherosclerosis in ESRD patients (4). Therefore, it is worthwhile to try the administration of free radical scavengers to reduce oxLDL- or phosphatidylcholine hydroperoxide–induced vasoconstriction and to slow down the progression of atherosclerotic vascular disease.

Vitamin C could reduce oxLDL-induced leukocyte adhesion to microvascular and macrovascular endothelium in vivo (29) and oxLDL-enhanced vascular smooth muscle cell apoptosis (30). Besides, vitamin C acts to protect plasma lipid against peroxidation at doses of $\approx 0.5$ to $10$ g/d (31). However, vitamin C could increase plasma oxalate, crystalluria, and urolithiasis in patients with renal dysfunction (31, 32). On the other hand, catechins act as antioxidants in vitro by scavenging ROS and chelating redox-active transition metal ions (33). They may also function indirectly as antioxidants through $I)$ the inhibition of redox-sensitive transcription factors, NF-κB, and activated protein 1 (19, 33); $2)$ the inhibition of “prooxidant” enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases, and xanthine oxidase; and $3)$ the induction of phase II and antioxidant enzymes, such as glutathione $S$-transferases and superoxide dismutases (34). Catechins inhibit the oxLDL-mediated induction of ICAM-1 and vascular cell adhesion molecule 1 expressions and decrease the influx of leukocytes in the vasculature by down-regulating the expression of E-selectin, decreasing IL-1 secretion from monocytes, and attenuating the activation of NF-κB (33). The present study suggests that extra supplementation of catechins to ESRD patients could protect against oxidative stress by scavenging hemodialysis-enhanced ROS (4). For the responses in palliating hemodialysis-enhanced hydrogen peroxide production, we found that an intake of 1.15 catechins (455 mg/397 g molecular weight calculated from 6 ingredients in the extract) was approximately equivalent to 2.84 mmol ascorbic acid (500 mg/176 g, molecular weight). Nevertheless, we showed that catechins had a superior effect on scavenging hypochlorous acid activity and preserving paraoxonase 1 activity, which, in turn, may counteract oxLDL-induced vascular injury.

Circulating proinflammatory cytokines and soluble apoptosis mediators are polypeptide mediators that have been associated with the activation of numerous functions, including the immune system and inflammatory responses. They are often overexpressed in patients with cardiovascular disease events (35). We used multiple cytokine arrays to quantify plasma proinflammatory, antiinflammatory, and soluble apoptosis mediators. Our data show that the dietary catechins down-regulate soluble apoptosis mediators (Fas/TNF receptor superfamily, member 6, Fas ligand), proinflammatory mediators (IL-6R, IL-8, and NAP-2), and antiinflammatory mediators (IL-1ra, sTNF-RI, and sTNF-RII) at the peptide concentration in posthemodialysis plasma. Moreover, our findings in the present study indicate that hemodialysis evokes several immunomodulatory mediators and that cytokine array data provide new insight into the molecular events by which catechin supplementation can exert immunomodulatory effects to improve the proinflammatory status of ESRD patients undergoing hemodialysis.

A previous study by our group showed an increase in the plasma concentrations of several proinflammatory cytokines, such as TNF-α, IL-6, IL-6R, and IL-8, and NAP-2, in ESRD patients undergoing chronic hemodialysis (4), which confirms the hypothesis of renal dysfunction associated with proinflammatory reaction. In ESRD patients, the synthesis and release of IL-6R (an antagonistic receptor) are significantly increased (36). The increased level of IL-6R release may partially counteract the inflammatory effects caused by IL-6 (37). However, IL-6R also promotes chemokine and adhesion molecule expressions through complex signaling of IL-6R and IL-6 during acute inflammation (38). The cytokines NAP-2 and IL-8 activate neutrophils and elicit selective diapedesis of PMNs into the extracellular space (39, 40). Most likely, this finding results from hemodialyzer-induced activation of monocytes, neutrophils, and tissue macrophages. However, other antiinflammatory mediators, such as IL-1ra, sTNF-R1, and sTNF-RII, are also up-regulated in ESRD patients undergoing hemodialysis. IL-1ra concentrations have been shown to increase during experimental endotoxemia (41), and IL-1ra inhibits IL-1 action by competitive binding to the IL-1 receptor (42). Granulocytes are a major source of IL-1ra (36), and, accordingly, they are the most likely source of the increased IL-1ra release in whole-blood cultures. The observed increase of IL-1ra expression is compatible with a state of neutrophil activation as documented in terms of enhanced activity for phagocytosis (43) or production of ROS (44). Thus, the relative increase of cytokine release in granulocytes may be greater after hemodialysis treatment. When present in sufficient amounts, sTNF-R1 and sTNF-RII may exert their antiinflammatory action by neutralizing TNF-α (45). Nevertheless, because IL-1ra and sTNFR are released in the monocyte response to local IL-1 and TNF-α production, it has been speculated that the increased appearance of IL-1ra and sTNFR may serve as “footprints” for monocyte activation (46). Therefore, in the present study, a decrease in TNF-α and a reduction in soluble TNF receptors and IL-1ra by catechins may be interpreted as an inhibitory effect on hemodialysis-enhanced leukocyte activation, rather than a proinflammatory effect. In the long-term effect, 3
mo of catechin supplementation significantly reduced the production of hydrogen peroxide and hypochlorous acid and concentrations of phosphatidylcholine hydroperoxide, CRP, sICAM-1, MCP-1, and TNF-α. We therefore presume that catechin supplementation, via antioxidant, antiatherosclerotic, and antiinflammatory reactions, attenuates hemodialysis-enhanced PMN activity to release several immunomodulatory mediators. In conclusion, our findings show that supplementation with decaffeinated green tea extracts (catechins) could be effective in reducing hemodialysis-induced ROS and palliating the subsequent adverse events—atherosclerosis and proinflammation.

The authors’ responsibilities were as follows—S-PH, M-SW, C-CY, and C-TC: conceived the hypothesis; S-PH, K-CH, and C-TC: conducted the statistical analyses; S-PH and C-TC: drafted the manuscript; M-SW and S-MH: contributed to the discussion of the results; C-CY and S-YL: contributed to the design and conduct of the study; and all authors: critically revised the manuscript. S-PH and M-SW contributed equally to this work. None of the authors had any conflict of interest.

REFERENCES


capacities to bind and chemoattract 293 cells transfected with either IL-8 receptor type A or type B. Cytokine 1997;9:37–45.


