Protection against Nitric Oxide Toxicity by Tea

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It is found that green tea and black tea are able to protect against nitric oxide ($NO^{\bullet}$) toxicity in several ways. Both green tea and black tea scavenge $NO^{\bullet}$ and peroxynitrite, inhibit the excessive production of $NO^{\bullet}$ by the inducible form of nitric oxide synthase (iNOS), and suppress the LPS-mediated induction of iNOS. The $NO^{\bullet}$ scavenging activity of tea was less than that of red wine. The high activity found in the polyphenol fraction of black tea (BTP) could not be explained by the mixed theaflavin fraction (MTF) or catechins [epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate (EGCG)], which were tested separately. Synergistic effects between the compounds, or the presence of a potent, unidentified $NO^{\bullet}$ scavenger, may explain the high activity of BTP. The peroxynitrite scavenging of tea was comparable to that of red wine. The main activity was found in the polyphenol fraction. MTF and the catechins were found to be potent peroxynitrite scavengers. Tea and tea components were effective inhibitors of iNOS. Of the tea components tested, only MTF had an activity higher than that of the tea powders. The polyphenol fractions of tea were much more active than the tea powders in suppressing the induction of iNOS. On the basis of its abundance and activity, EGCG was the most active inhibitor. The protective effect of tea on $NO^{\bullet}$ toxicity is discussed in relation to the beneficial effect of flavonoid intake on the occurrence of cardiovascular heart disease.

Keywords: *Tea; flavonoids; nitric oxide; peroxynitrite; catechin; theaflavin; epigallocatechin*

INTRODUCTION

The role of nitric oxide ($NO^{\bullet}$) in physiology is ambiguous. On the one hand, pivotal physiological functions are executed by $NO^{\bullet}$, for example, the control of blood pressure by its action as an endothelial derived relaxing factor (Moncada et al. 1991). On the other hand, $NO^{\bullet}$ is toxic, predominantly due to the formation of peroxynitrite formed in the reaction with superoxide radicals ($O_2^{\bullet -}$) (Rubbo et al., 1996; Radi et al., 1991).

Peroxynitrite is able to initiate various toxic processes. For example, peroxynitrite is able to oxidize low-density lipoproteins (LDL), a key event in the etiology of arteriosclerosis (Graham et al., 1993; Steinberg et al., 1989). The major source of peroxynitrite in arteriosclerosis is inflammatory cells. Activated inflammatory cells contain an NADPH-oxidase and the inducible form of nitric oxide synthase (iNOS). These enzymes mediate the massive production of superoxide anion radicals and nitric oxide radicals, the ingredients needed for the formation of peroxynitrite (Radi et al., 1991).

Previously, it has been shown that flavonoids, that is, a group of polyphenolic antioxidants, are able to reduce peroxynitrite toxicity. The flavonoids scavenge the precursors of peroxynitrite, that is, the superoxide anion radical (Ricardo da Silva et al., 1991; Sato et al., 1996) and the nitric oxide radical (Haenen and Bast, 1999). Moreover, the flavonoids are efficient scavengers of peroxynitrite itself (Haenen et al., 1997). The prevention of peroxynitrite toxicity might provide a rationale for the reduction of cardiovascular heart disease (CHD) that is associated with a high content of flavonoids in the diet (Hertog et al., 1993).

A major source of flavonoids in the Western diet is tea. The most frequently consumed teas are black tea and green tea. In the production of green tea, steaming and drying of the tea leaves prevent oxidation of the polyphenols in the leaves. The polyphenols account for up to 30% of the dry weight of green tea. The major components are catechins, mainly epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Enzymatic, aerobic oxidation of leaf polyphenols, and subsequent condensation—a process known as fermentation—takes place in the production of black tea. Due to the fermentation, the total catechin content is reduced—black tea contains ~20−30% of the total catechin content of green tea—and new products, such as theaflavins, are formed. The theaflavins comprise 0.3−2% of the dry weight of black tea. The major theaflavins are theaflavin, theaflavin 3-gallate, theaflavin 3′-gallate, and theaflavin 3,3′-digallate. The chemical structures of the most important catechins and theaflavins are depicted in Figure 1.

In the present study, the ability of tea to interact with the $NO^{\bullet}$ system is examined. For both black tea and green tea the $NO^{\bullet}$ and the peroxynitrite scavenging activities and the ability to inhibit iNOS are determined. In an attempt to identify the compounds that are responsible for the effects, various tea fractions and flavonoids present in tea are also studied.

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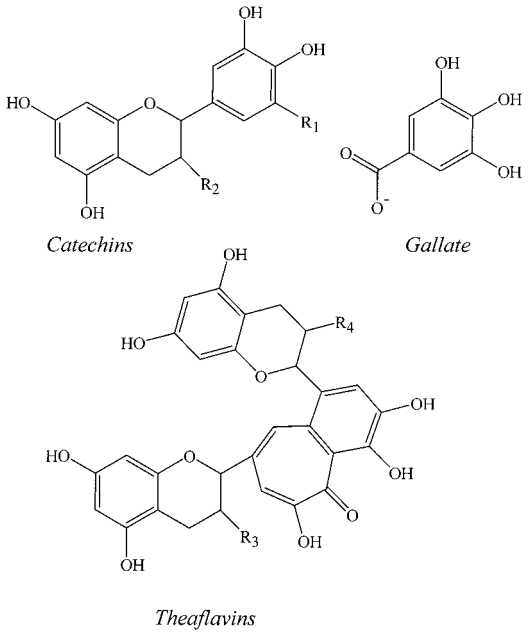

Figure 1. Structures of the catechins and theaflavins. For EC R_1 = H and R_2 = OH; for EGC R_1 = OH and R_2 = OH; for ECG R_1 = H and R_2 = gallate; for EGCG R_1 = OH and R_2 = gallate. For theaflavin, R_3 = R_4 = OH; for theaflavin-3-monogallate R_3 = gallate and R_4 = OH; for theaflavin-3′-monogallate R_3 = OH and R_4 = gallate; for theaflavin-3,3′-digallate R_3 = R_4 = gallate.

MATERIALS AND METHODS

Chemicals. Black tea powder, green tea powder, and tea fractions were gifts from Lipton (Englewood Cliffs, NJ) Their composition is given in Table 1. The amount of solids obtained from tea leaves is variable, depending, for example, on the size of the leaf, brewing time, and agitation. The average amount of dry powder obtained from an English cup is 2−5 g/L. For calculation of the activity of tea, 3 g of solids/L was used. Epicatechin was from Aldrich (Zwijndrecht, The Netherlands).

Peroxynitrite was synthesized in a quenched flow reactor, as previously described (Radi et al., 1991). Excess hydrogen peroxide was removed by MnO_2 treatment.

RPMI 1640 medium, HAM's F-12 medium, glutamine, and Fungibact were obtained from BIOWhitaker (Walkersville, MD). Fetal calf serum (FCS) was from Life Technologies (Paisley, U.K.). [^{3}H]-L-Arginine was obtained from Amersham Corp. (Buckinghamshire, U.K.), and LPS was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical purity grade.

NO Scavenging. NO$^\bullet$ scavenging was determined according to the method of Vriesman et al. (1997). Briefly, deoxygenated water was purged with NO$^\bullet$ gas for ∼1 min. Two microliters of the NO$^\bullet$-spiked water was added to 20 mL of 50 mM phosphate buffer (pH 7.4) in a thermostated test vessel (37 °C). During measurements the test vessel was kept under an N_2 atmosphere. The NO$^\bullet$ concentration was monitored with an Iso-NO meter (World Precision Instruments, Sarasota, FL), which was coupled to a MacLab interface (ML020 MacLab/8, ADInstruments, London, U.K.). The decrease in NO$^\bullet$ concentration was followed in time in the presence or absence of the test compound in solution. The stock solution of the test compound was incubated at 37 °C for 10 min before use. The (pseudo) first-order reaction constant was calculated.

Peroxynitrite Scavenging. Peroxynitrite scavenging was measured by the oxidation of dihydrorhodamine 123, as described by Kooy et al. (1994). Fluorescence measurements were performed on a Shimadzu RF-5001 PC fluorometer with excitation and emission wavelengths of 500 and 536 nm, respectively, at 37 °C. The effects are expressed as the concentration giving 50% inhibition of the oxidation of dihydrorhodamine 123 (IC_{50}).

Induction of iNOS. A rat pulmonary macrophage cell line, NR8383 (kindly provided by Dr. R. J. Helmke, Department of Pediatrics, University of Texas, Health Science Center, San Antonio, TX) was used. Cells were maintained in culture at a floating cell concentration of 10^5 cells/mL in RPMI 1640 medium containing 2 mM glutamine, 0.5% Fungibact (50 units/mL penicillin, and 50 μg/mL streptomycin), and 10% heat-inactivated FCS. Cells were grown in a humidified 5% CO_2 incubator at 37 °C. Cells were collected (1500 rpm, 5 min) and resuspended at a cell concentration of 10^6 cells/mL in HAM's F-12 medium containing 2 mM glutamine and 0.5% Fungibact. iNOS was induced by the addition of 10 μg/10^6 cells of bacterial LPS. After 24 h, the cells were harvested. To assess the effect of test compounds on iNOS induction, the test compounds (0.5 mg/mL) were present during the LPS treatment.

Determination of iNOS Activity. iNOS activity was determined by quantifying the amount of [^{3}H]-L-citrulline that is formed from [^{3}H]-L-arginine (Bredt and Snyder, 1990). LPS-treated cells were collected and resuspended at a cell concentration of 10^7 cells/mL in Tris-HCl buffer (50 mM, pH 7.4) containing 2 μM leupeptin, 1 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol, 10 μg/mL trypsin inhibitor from soybean, 2 μg/mL aprotinin, 0.1 mM EDTA, and 320 mM sucrose. The cell suspension was subsequently sonificated for 5 min. iNOS activity was started by adding 40 μL of the obtained cell suspension to 60 μL of a Tris-HCl buffer (50 mM, pH 7.4) containing 1 mM NADPH, 10 μM L-arginine, and 20 nCi of [^{3}H]-L-arginine (specific activity = 63 Ci/mmol). To selectively measure iNOS, a calcium-free buffer was used and EGTA was present in a final concentration of 1 mM. Nonenzymatic conversion was determined by using a heat-inactivated cell suspension (2 min at 100 °C). After 15 min of incubation at 37 °C, the reaction was terminated by adding 1 mL of ice-cold HEPES buffer (20 mM, pH 5.5) and putting the incubation mixture on ice. L-Arginine was removed by adding 1 mL of a Dowex ion exchange suspension (a 50% suspension of Dowex-50-W, Na^+ form, 200−400 mesh, 8% cross-linking in water, pH 7). After separation of the Dowex by centrifugation, the amount of [^{3}H]-L-citrulline in the supernatant was measured. To assess the effect of the compounds on iNOS activity, the compounds were present during the incubation with [^{3}H]-L-arginine.

Determination of Nitrite/Nitrate Production. Nitrite and nitrate production was determined in cells (10^6 cells/mL) stimulated for 24 h with LPS (10 μg/mL) in the absence or presence of the compounds (0.5 mg/mL). Protein was removed from 150 μL of the cell suspension by adding 30 μL of NaOH solution (1 mM) and 30 μL of $ZnSO_4$ solution (1.3 mM). After vigorous shaking, the incubations were left on ice for 5 min and centrifuged (5 min, 13000*g* at 4 °C). For the conversion of nitrate to nitrite, 100 μL of the supernatant was added to 50 μL of a *Klebsiella pneumoniae* solution. The *K. pneumoniae* solution (0.7 mg of protein/mL) contained 0.33 M sodium formate and 0.13 M *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (pH 7.4).

Nitrate reduction was performed at room temperature in a vacuum desiccator. After 30 min, 0.5 mL of nitrogen-purged water was added. After centrifugation, 0.5 mL of the supernatant was added to 50 μL of a sulfanyl amide solution (1% in 5% H_3PO_4 solution). After mixing, 50 μL of 0.1% *N*-(1-naphthyl)ethylenediamine was added, and within 30 min the absorption at 540 nm was determined. Standards containing 0−150 μM $NaNO_2$ and $NaNO_3$ were used for calibration.

RESULTS

NO$^\bullet$ Scavenging. Green tea was found to be a more efficient scavenger of NO$^\bullet$ than black tea; green tea was ∼5 times more potent than black tea (Table 2). Taking an average amount of 3 g/L of tea, it can be calculated that green tea has an activity comparable to that of white wine and is ∼50 times less active than red wine (Verhagen et al., 1996).

The polyphenol fraction accounted for the major part of the NO$^\bullet$ scavenging by green tea (Table 2). Compared

Table 1. Composition of the Teas, Tea Fractions, and Catechins Used in This Study[a]

	green tea	black tea	GTP	BTP	EC	EGC	ECG	EGCG	MTF
catechins	*32.8*	*6.80*	*79.4*	*39.0*	*88.7*	*66.3*	*73.1*	*89.4*	
(+)-catechin	1.3	0.32	1.7	1.9	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
(+)-gallocatechin	1.4	0.28	2.4	0.80	<LLOQ	1.86	<LLOQ	<LLOQ	<LLOQ
(+)-gallocatechin gallate	0.39	<LLOQ	<LLOQ	1.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
EC	4.2	0.79	9.8	5.6	88.7	2.4	<LLOQ	<LLOQ	<LLOQ
EGC	7.9	1.2	8.6	1.6	<LLOQ	58.4	<LLOQ	<LLOQ	<LLOQ
ECG	5.8	1.4	13.2	10.6	<LLOQ	<LLOQ	72.2	1.8	<LLOQ
EGCG	11.8	2.8	43.7	17.5	<LLOQ	4.0	0.89	87.5	<LLOQ
theaflavins		*1.18*		*12.9*					*94.1*
theaflavin	<LLOQ	0.36	<LLOQ	4.2	<LLOQ	<LLOQ	<LLOQ	<LLOQ	21.4
theaflavin 3-gallate	<LLOQ	0.36	<LLOQ	4.1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	29.9
theaflavin 3′-gallate	<LLOQ	0.19	<LLOQ	1.8	<LLOQ	<LLOQ	<LLOQ	<LLOQ	15.3
theaflavin 3,3′-digallate	<LLOQ	0.27	<LLOQ	2.8	<LLOQ	<LLOQ	<LLOQ	<LLOQ	27.5
gallic acid	0.15	0.80	0.80	4.5	<LLOQ	1.0	<LLOQ	<LLOQ	<LLOQ
caffeine	6.4	6.6	<LLOQ	0.27	<LLOQ	2.0	<LLOQ	<LLOQ	<LLOQ
theobromine	0.25	0.25	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
unknown material	60.5	84.4	19.8	37.4	11.3	30.3	26.9	10.6	5.9

[a] Results are represented as percentage (w/w); <LLOQ indicates the value is below the lower limit of quantification.

Table 2. Effects of Teas, Tea Fractions, and Catechins on the NO• System

compound[a]	NO• scavenging (mL mg^{-1} s^{-1})	peroxynitrite scavenging (IC_{50}, μg mL^{-1})	iNOS inhibition (IC_{50}, mg mL^{-1})	nitrate/nitrite production[b] (%)	iNOS induction[b] (%)
black tea	0.019 ± 0.009	0.882 ± 0.036	0.24 ± 0.07	35 ± 5	51 ± 4
green tea	0.101 ± 0.016	0.380 ± 0.037	0.47 ± 0.04	15 ± 6	20 ± 4
BTP	0.447 ± 0.031	0.232 ± 0.015	0.27 ± 0.06	2 ± 2	1 ± 1
GTP	0.207 ± 0.019	0.171 ± 0.014	0.51 ± 0.07	1 ± 0	3 ± 2
MTF	0.368 ± 0.027	0.331 ± 0.006	0.13 ± 0.03	3 ± 2	2 ± 1
EC	0.053 ± 0.004	0.181 ± 0.010	>1	65 ± 10	31 ± 6
EGC	0.134 ± 0.017	0.228 ± 0.005	1.0 ± 0.2	35 ± 7	20 ± 6
ECG	0.194 ± 0.017	0.172 ± 0.003	0.41 ± 0.05	3 ± 2	10 ± 1
EGCG	0.167 ± 0.023	0.151 ± 0.005	0.93 ± 0.05	0 ± 2	1 ± 1

[a] Composition of the tea fractions is given in Table 1. [b] Compounds were used in a concentration of 0.5 mg/mL. Results are expressed as percentage of the control value.

to other flavonoids, the catechins have an average NO• scavenging activity (Haenen and Bast, 1999). The activity of the catechins tested was similar to or lower than that of GTP. This means that GTP must contain other compounds that have a higher NO• scavenging activity than the catechins studied.

The polyphenol fraction of black tea showed a high activity. On the basis of the results, the major contributors to the NO• scavenging of black tea have to be found in the polyphenol fraction. MTF shows a higher activity than the catechins, although the activity of MTF does not exceed that of BTP. Synergistic effects between the compounds, or the presence of a potent, unidentified NO• scavenger, may explain the high activity of BTP.

As also stated above, NO• has an ambiguous role: It controls various important physiological activities but it may also give rise to the toxic peroxynitrite. NO• scavenging may therefore not only affect NO toxicity but also intervene in the desirable effects of NO•.

Peroxynitrite Scavenging. Comparable to the NO• scavenging, green tea is also the better peroxynitrite scavenger; green tea is >2 times as active as black tea. Taking an average of 3 g/L of powdered tea, it can be calculated that green tea has an activity similar to that of red wine and 25 times higher than that of white wine (Paquay et al., 1997). The high activity of the polyphenol fractions indicates that the major peroxynitrite scavengers of black tea and green tea are found in these fractions. Previously, it has been shown that also in wine the polyphenols are predominantly responsible for the peroxynitrite scavenging activity (Paquay et al., 1997). In addition, it has been reported that EGCG is a far better peroxynitrite scavenger than ascorbate or glutathione (Fiala et al., 1996). Pannala et al. (1997) reported that for catechins the rank order in potency was ECG ≥ EGCG > EC ≥ EGC. In the present study a comparable rank order was found, although the differences in activity were small. A previous study showed that probably more than one "pharmacophoric" group can be found in flavonols. Both the catechol group (ring B) and the hydroxyl group at position 3 seem to be important (Haenen et al., 1997). On the basis of the results we obtained in the present study, the pharmacophoric groups in the catechins studied are similar or have a similar activity. As shown in Table 2, the theaflavins seem to be less active than the catechins in peroxynitrite scavenging. It should be noted, however, that the activities are given per gram of compound. When the activity is expressed on a molar basis, the MTF have an activity comparable to that of the catechins. Apparently, catechin and theaflavin molecules contain comparable active pharmacophore(s).

iNOS Inhibition. The ability of tea and tea components to inhibit iNOS of LPS-treated, homogenized cells was also tested. It was found that tea and tea components are effective inhibitors of iNOS (Table 2). The activity of some of the test compounds was higher than that of the commonly used NOS inhibitor L-NAME, that has an IC_{50} of 0.8 mg/mL (0.34 μmol/L). Black tea appeared to be twice as active as green tea. Of the tea fractions tested, MTF was the most active. The higher activity of BTP compared to GTP can partially be

explained by the contribution of MTF in BTP. MTF comprises ~13% of the identified polyphenols in BTP and is not present in GTP. The catechins EC, EGC, EGC, and EGCG were less active than GTP. Apparently, ingredients of GTP other than the catechins are responsible for the inhibition of iNOS by GTP. Of the tea components tested, only MTF had an activity higher than those of the tea powders. Apparently, other ingredients of black tea and green tea also possess a potent inhibitory activity.

Recently, Chan et al. (1997) also studied the effect of tea catechins on iNOS inhibition. They suggested that iNOS inhibition by the catechins is due to the inhibition of the binding of the substrate L-arginine and the cofactor tetrahydrobiopterin by the catechins. In their study the rank order of potency in iNOS inhibition was ECG > EGCG > EGC. In the present study, the same rank order for these catechins in iNOS inhibition was found. In addition, we found that EC had the lowest potency of the catechins tested. The low activity of EC is in line with the suggestion of Chan et al. (1997) that the gallate structure may play a critical role in the inhibition because EC lacks this structure (Figure 1). It was found that MTF exceeds the activity of the most active catechin. Three of the four theaflavins contain a gallate moiety; one of them even has two (Table 1). This might explain the high activity of the MTF fraction.

Nitrite and Nitrate Production. The reduction of iNOS activity was also tested on intact cells. In these experiments $NO^{\bullet}$ production was quantified by measuring nitrite and nitrate production. During the activation of the cells by LPS, the tea and tea components were present. It was found that also in this assay, the tea and tea components were active inhibitors (Table 2). Green tea was approximately twice as active as black tea. The activity of the polyphenol fractions was much higher than that of the teas, indicating that the major activity of the teas resides in these fractions. Previously, it has been demonstrated that EGCG inhibits LPS and interferon-γ-induced nitrite production by mouse peritoneal cells (Lin and Lin, 1997). In the present study, we found that ECG and EGCG showed the highest activity of the catechins tested. Taking their relative abundance also into account, ECG and EGCG are responsible for the main activity found in GTP and green tea. MTF had an activity comparable to that of ECG and EGCG. This indicates that MTF also has a major contribution to the effect of black tea and BTP on the accumulation of nitrite and nitrate from LPS-stimulated macrophages.

iNOS Expression. There is a difference in rank order of activity of the compounds tested when they are added either during or after LPS treatment. EGCG is a relatively poor inhibitor of iNOS activity, whereas it is—besides EGC and MTF—the most active inhibitor of nitrite and nitrate production by the macrophage cells. The most likely explanation for this discrepancy is an effect of EGCG on the induction of iNOS. To test this hypothesis, cells were incubated with LPS in the presence of the tea and tea components. After this treatment, the cells were harvested and washed, and the iNOS activity was determined. In this assay again a high activity of tea and tea components was found (Table 2). EGCG almost completely blocked the induction of iNOS by LPS in the cell line examined. This indicates that the above-mentioned discrepancy between the effect of EGCG on the inhibition of iNOS and the effect of EGCG on the accumulation of nitrite and nitrate can indeed be explained by the inhibition of iNOS induction by EGCG.

In the inhibition of the induction of iNOS, green tea was twice as potent as black tea. The polyphenol fractions of black and green tea were much more active than the tea powders, indicating that the polyphenol fractions of the teas contain the most active compounds. The catechins were efficient inhibitors. The most active compound is EGCG. The difference in catechin content between black tea and green tea is reflected in their effects on iNOS induction. The catechin content of green tea (~32%) is 4−5 times as high as that of black tea (~7%). In fact, the high EGCG content (~12%) can explain the greater part of the inhibition of iNOS induction by green tea. Recently, Chan et al. (1997) and Lin and Lin (1997) have reported that catechins present in tea are able to block NF-κB, a transcription factor necessary for iNOS induction. Lin and Lin (1997) have reported that the rank order for this activity was EGC > EGCG > EC > ECG. In the present study, another rank order for the inhibition of iNOS induction was found, that is, EGCG > ECG > EGC > EC. This difference in rank order may be explained by an effect of the catechins on processes other than NF-κB activation involved in iNOS induction. There are several possibilities, including a reduction of protein synthesis and an effect on mRNA breakdown. In addition, the use of different cells may have caused the difference in rank order.

Besides the catechins, the theaflavins are also effective inhibitors of iNOS induction. In the production of black tea, the relatively low amount of theaflavins formed during the fermentation process (~1% of the total weight) does not compensate for the loss of the catechins (~26%). On the basis of both its activity and content, EGCG appeared to be the major inhibitor of iNOS induction not only in green tea but also in black tea. The results of the present study indicate that, besides the catechins studied and the theaflavins, black tea may contain (an) other unidentified ingredient(s) that prevent the LPS-induced expression of iNOS.

DISCUSSION

The process of arteriosclerosis has various characteristics of a local inflammation. Inflammatory cells become activated and are attracted to the site of arteriosclerosis. In the final process of arteriosclerosis, free radicals play a pivotal role. Several speculations have been made on the etiology of arteriosclerosis. The hypothesis that arteriosclerosis has a viral or bacterial trigger originating from, for example, chronic periodontal Gram-negative infections or pulmonal *Chlamydia* infections, has gained much attention (Steinberg et al., 1989; Beckman et al., 1994). These focal infections provide a pathological burden of mediators such as LPS, which serves to initiate and exacerbate arteriosclerosis. One of the processes initiated by LPS is the coordinated expression of multiple inflammatory genes encoding for, for example, adhesion molecules, cytokines, and enzymes such as iNOS. In addition, inflammatory cells contain a membrane-bound NADPH-oxidase that produces superoxide anions. The combined formation of superoxide anions and nitric oxide from NADPH-oxidase and iNOS results in the formation of peroxynitrite. The role of peroxynitrite in arteriosclerosis has previously been studied. Extensive peroxynitrite production in arterio-

sclerotic lesions has been demonstrated (Beckman et al., 1994). Moreover, it is known that peroxynitrite is able to oxidize LDL, a key process in artheriosclerosis (Graham et al., 1993; Steinberg et al., 1989).

As stated previously, the molecular basis for the protective effect of flavonoids on CHD may be found in the prevention of peroxynitrite toxicity. It has been shown that flavonoids efficiently scavenge peroxynitrite (Haenen et al., 1997), nitric oxide (Haenen and Bast, 1999), and the superoxide anion (Facino et al., 1994; Ricardo da Silva et al., 1991; Sato et al., 1996). In the present study it has been shown that tea and tea components may intervene with $NO^{\bullet}$ toxicity in several ways. Not only are tea and tea components scavengers of $NO^{\bullet}$ and peroxynitrite, they also inhibit the activity of iNOS and inhibit the induction of iNOS by LPS. In the inhibition of NOS activity, the theaflavins are the most active compounds. In the inhibition of iNOS induction, the theaflavins and catechins have comparable activities. Taking the relative abundance into account, EGCG seems to be the most important ingredient of the teas in the defense against $NO^{\bullet}$ toxicity. Protection by tea against the LPS-induced effects nicely fits the above-mentioned hypothesis that mediators such as LPS originating from focal infections are the initial trigger for arteriosclerosis. By blocking the LPS effects, tea intervenes at an early stage in artheriosclerosis.

Extrapolation of the in vitro results obtained in the present study to a protective effect on CHD is complicated. Data on the bioavailability and the distribution of the tea components are scarce (Hollman et al., 1997). However, tea consumption results in an increase of the antioxidant capacity of blood plasma (Facino et al., 1994). In addition, it has been reported that flavonoids accumulate between the endothelial layer and vascular smooth muscle cells, exactly the site where arteriosclerosis occurs (Neuman et al., 1992). Moreover, a reduced incidence of CHD has been associated with a high flavonoid intake (Hertog et al., 1993; Facino et al., 1994), and tea provided approximately half of the measured flavonoid intake. In the etiology of CHD, massive production of superoxide anion and nitric oxide radicals by inflammatory cells results in peroxynitrite toxicity. The ability of tea to protect against this toxicity seems to be of great interest. As shown in the present study, both black tea and green tea are able to offer protection in several ways.

ABBREVIATIONS USED

GTP, green tea polyphenols; BTP, black tea polyphenols; MTF, mixed theaflavin fraction; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; iNOS, inducible form of nitric oxide synthase; IC_{50}, concentration giving 50% inhibition; CHD, cardiovascular heart disease.

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