Prevention of Hypertension, Cardiovascular Damage and Endothelial Dysfunction with Green Tea Extracts

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Background: We investigated the effect of green tea extract (GTE) in arterial hypertension with high oxidative stress. Angiotensin (Ang) II induces endothelial dysfunction (ED) that is crucial for the development of atherosclerosis and hypertension.

Methods: Male Sprague-Dawley rats, 13 weeks old, randomly assigned to drinking water with or without GTE (6 mg/mL) received a vehicle, a high (700 μg/kg/d) or a low (350 μg/kg/d) Ang II dose for 13 days, by osmotic mini-pumps. Blood pressure (BP) was measured with telemetry. After sacrifice, left ventricular (LV) mass index, small mesenteric artery media-to-lumen ratio, and concentration–response curves of phenylephrine-precontracted arteries to acetylcholine were evaluated. The effect of the superoxide dismutase (SOD-1) analog tempol on artery responses to acetylcholine was assessed. Oxidative stress was measured by plasma hydroperoxides and nitrotyrosine levels. The mRNA of heme oxygenase 1 (HO-1), NADPH oxidase endothelial p22^{phox} subunit, and SOD-1 was also measured in the aorta.

Results: Compared with vehicle high Ang II increased BP, LV mass index, media-to-lumen ratio, and hydroperoxide radicals. The GTE blunted these increases, prevented the increase in HO-1, p22^{phox}, and SOD-1 mRNA in aorta caused by Ang II, and reduced them below baseline levels. Low Ang II dose increased BP values and plasma hydroperoxides only during the first week. Both Ang II doses shifted rightward the curves to acetylcholine; this was prevented in vivo by GTE and abolished in vitro by tempol.

Conclusions: The GTE prevented hypertension and target organ damage induced by a high Ang II dose, likely by prevention or scavenging of superoxide anion generation.   Am J Hypertens 2007;20:1321–1328 © 2007 American Journal of Hypertension, Ltd.

Key Words: Green tea, endothelium, hypertension, angiotensin II.

Endothelial dysfunction (ED) plays a role in the triggering and progression of cardiovascular (CV) disease and predicts morbid events.[1,2] Thus, the maintenance of a healthy vascular endothelium is a major goal of CV prevention.

Nutrients, such as alcohol-free wine extract, quercetin,[3] dark chocolate,[4] and green tea (GT),[5] can exert protective effects on the endothelium. Epidemiologic studies also indicate that GT consumption confers CV protective effects.[6]

These favorable effects can be attributed, at least in part, to the antioxidant properties and vasodilating effects[7] of the GT catechins, but this remains contentious.[8] Whether GT can protect from the development of arterial hypertension (HT), ED, and HT-induced target organ damage through its antioxidant properties also remained unclear.

We therefore investigated the mechanisms of GT extract on CV protective effects in a model of arterial HT with high oxidative stress.

Methods
Chemicals

Lyophilized GT extract containing 59% epigallocatechin-3-gallate (EGCG), 86% total catechins, and 0.5% caffein

was supplied by SOFAR (Trezzano Rosa, Milan, Italy). Human angiotensin (Ang) II acetate and other chemicals were purchased from Sigma (Saint Louis, MO).

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), 13 weeks of age (body weight 250 to 350 g), were divided into five groups of six animals each.

Telemetry Blood Pressure Measurements

The Dataquest IV telemetry system (Data Sciences International, Arden Hills, MN) was used for the direct measurement of systolic and diastolic blood pressure (BP), according to the manufacturer's instructions. The rats were placed individually in their cages, and were unrestrained and untethered.

Systolic and diastolic BP were monitored continuously at a sampling rate of 5 min during 24 h. After exclusion of outliers the mean daytime (rest time: 8 AM to 7:59 PM) and night-time values (activity time: 10 PM to 7:59 AM) were calculated.

Study Design

Seven days after surgery the rats were allocated to five treatment groups (Fig. 1): vehicle (0.9% NaCl; placebo group), high (Ang_H 700 μg/kg/d), or low (Ang_L 350 μg/kg/d) Ang II dose, was administered subcutaneously by osmotic mini-pumps (model 2ML2, 14-day infusion, Alzet, Palo Alto, CA). The Ang_H and Ang_L doses chosen were shown to induce oxidative stress and CV changes, including cardiac fibrosis,[9,10] and to determine ED and CV changes, respectively.[11,12] Rats consumed a normal sodium diet and either tap water or water containing GT extract, which was prepared fresh daily (6 mg/mL equivalent to 3.5 mg/mL EGCG). This dosage was chosen considering that: (1) catechin metabolism or conjugation to their inactive forms might be higher in rats than in humans[13,14]; (2) GT drinkers can safely have 10 to 15 cups/d[15,16]; (3) the GT effects that humans might experience with a lifelong consumption had to be investigated within an experimentally reasonable time period. The administration of GT extract (GTE) was initiated 48 h before mini-pump implantation in both Ang_H and Ang_L groups, hereafter named Ang_H+GTE and Ang_L+GTE. To exclude potential confounding effects due to inadequate Ang II delivery during the final day of 14-day infusion, rats were sacrificed on day 13. For biochemical measurements blood was collected from the tail vein at the end of the first week and the end of the experiment.

The protocol followed current guidelines (NIH publication no. 93-23, revised 1985) and was approved by the Institutional Animal Care and Use Committee.

Cardiac Hypertrophy

On day 13 the rats were anesthetized, weighed, killed, and the hearts were removed. The ratios of heart-to-body weight (cardiac mass index), left and right ventricle-to-body weight (LV and RV mass index) were determined.

Marker of Oxidative Stress and HO-1, p22^{phox}, and SOD-1 mRNA in the Aorta

To estimate oxidative stress and the effect of enhanced peroxynitrite generation on protein nitrosylation we measured the plasma hydroperoxides (alkoxyl and peroxyl radicals) (D-ROM, Diacron International Srl, Grosseto, Italy), and nitrotyrosine levels (Enzyme Immunoassay (OxisResearch, Portland, Oregon),[17] respectively.

Real-time reverse transcription (RT)–polymerase chain reaction (PCR) was used to measure the heme oxygenase 1 (HO-1; NM_012580), p22^{phox} (AJ295951), and superoxide (SOD-1; NM_017050) dismutase mRNA in the thoracic aorta, with the Universal ProbeLibrary Probes (Roche, Monza, Italy). Total RNA was isolated using RNeasy Mini Kit (Qiagen, Milan, Italy). One microgram of HO-1, p22^{phox}, and SOD-1 mRNA was reverse-transcribed and amplified in duplicate with specific primers with LightCycler 480 Probes Master (Roche). The mRNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM_017008) was similarly processed as a housekeeping gene to serve as a control for RT-PCR performance. Quantification of gene expression was carried out by comparative cycle threshold (Ct) ($2^{-\Delta\Delta Ct}$) method.[18]

Preparation of Isolated Microvessels

Third and fourth order branches of the mesenteric artery (150 to 350 μm diameter, 1 to 2 mm length) were isolated and mounted on glass micropipettes in a water-jacketed perfusion chamber (Living Systems Instrumentation, Burlington, VT) as described.[19] The intraluminal pressure was set at 100 mm Hg during the experiment.

Evaluation of Small Mesenteric Artery Response to Acetylcholine and Sodium Nitroprusside and Structure

The arteries were initially superfused with physiological salt solution (PSS) containing phenylephrine (PE) ($10^{-6.5}$ to $10^{-5.5}$ mol/L) at a concentration capable of giving a 50% to 70% contraction. The effect of increasing acetylcholine (ACh) dose (10^{-9} to 10^{-4} mol/L) was evaluated by vessel internal diameter variations. After washing, the tissues, re-exposed to PE and the effect of sodium nitroprusside (SNP, 10^{-6} to 10^{-4} mol/L) was evaluated. Concentration–response curves to ACh and to SNP were also repeated after 35 min of superfusion with tempol (10^{-3} mol/L).

The media-to-lumen ratio of the pressurized small mesenteric arteries was calculated as average of measures at six locations with an inverted light microscope (Eclipse TS100-F, Nikon, Tokyo, Japan) at an optical magnification of $\times$300.[20]
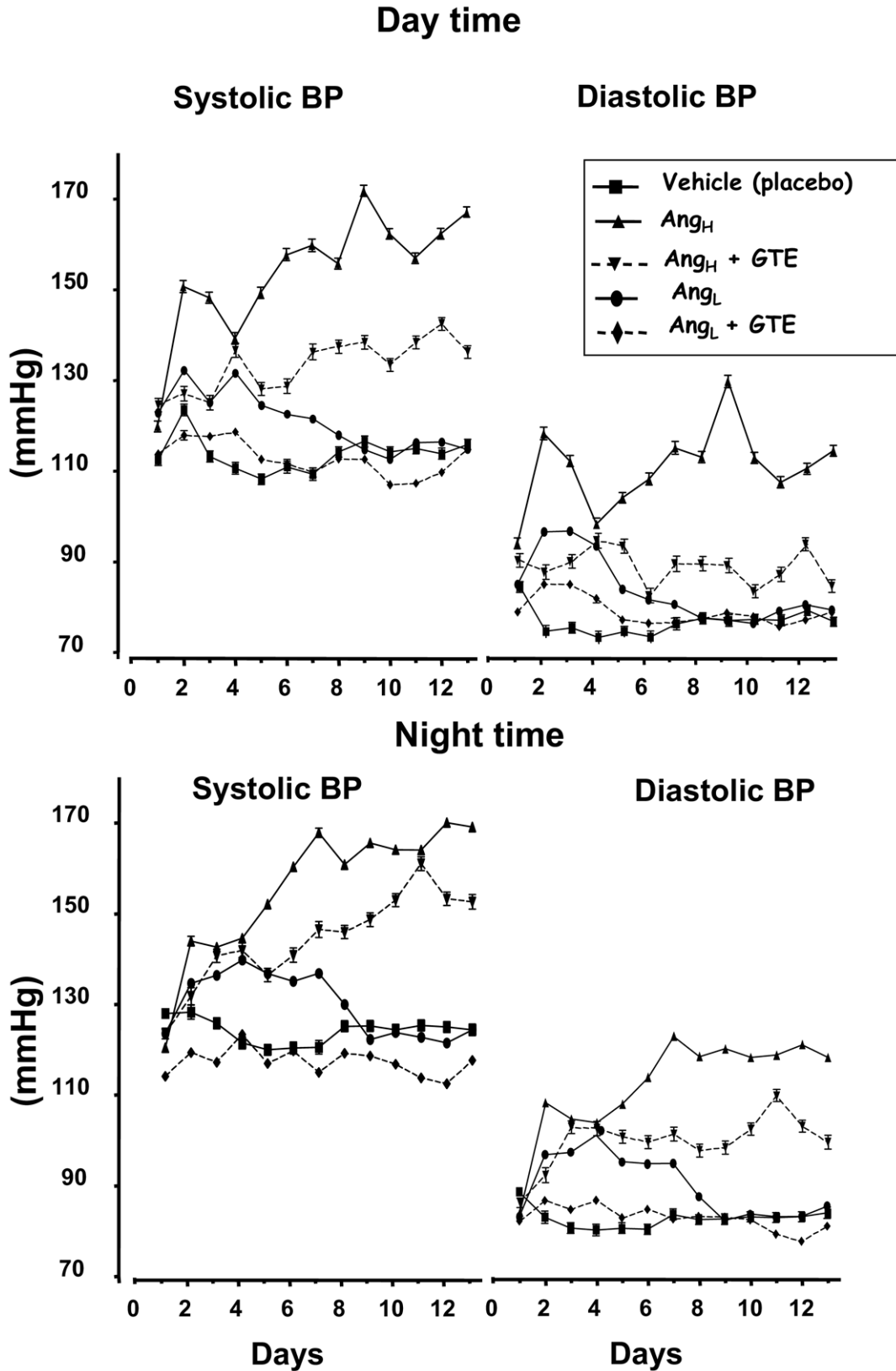
FIG. 1. Night-time and daytime systolic and diastolic BP of Sprague-Dawley rats, administered drinking tap water infused with a vehicle (■), Ang_H (▲), or Ang_L (●) dose. Parallel groups infused with Ang_H (▼) or Ang_L (♦) dose were given green tea extract (GTE) in drinking water. By day 2 of infusion, the Ang_H group showed significantly ($P < .001$) higher systolic and diastolic BP, in both night-time and daytime, compared to all other groups, in repeated-measure ANOVA and post-hoc test. In contrast, in the Ang_L group, the systolic and diastolic BP values were higher than in the placebo group only during week 1. The GTE significantly lowered both systolic and diastolic BP, compared with the Ang_H group, throughout the study period. They also abolished the pressor effect elicited by the low Ang II dose during week 1. See text for other abbreviations.

Table 1. Left (LV), right (RV), total heart weight, body weight, plasma nitrotyrosine levels, and aortic expression of HO-1, p22^{phox}, and SOD-1 genes in the five experimental groups

	Vehicle	(Placebo) Ang$_H$	Ang$_H$+GTE	Ang$_L$	Ang$_L$+GTE
LV weight (mg)	852 ± 41	1080 ± 23†‡*	998 ± 39§‡	813 ± 24	765 ± 20¶
LV mass index (mg/g)	1.86 ± 0.01¶	2.81 ± 0.06‖	2.43 ± 0.06‖	1.95 ± 0.03¶	1.88 ± 0.07¶
RV weight (mg)	196 ± 18	188 ± 15	239 ± 30	178 ± 9	188 ± 14
RV mass index (mg/g)	0.43 ± 0.04	0.49 ± 0.03	0.57 ± 0.006	0.43 ± 0.02	0.46 ± 0.01
Total heart weight (mg)	1048 ± 49	1269 ± 24‡§*	1237 ± 67‡	992 ± 31¶	953 ± 33¶
Cardiac mass index (mg/g)	2.34 ± 0.06¶	3.34 ± 0.05†‡	3.03 ± 0.11†‡	2.55 ± 0.06¶	2.53 ± 0.06¶
Body weight (g)	458 ± 24	384 ± 10	409 ± 9	412 ± 13	408 ± 19
Nitrotyrosine (nmol/L)	31.3 ± 0.8	30.3 ± 0.6	29.7 ± 1.1	27.9 ± 1.2	29.6 ± 2.3
HO-1 mRNA expression	0.70 ± 0.28	1.33 ± 1.20	0.11 ± 0.04	NA	NA
P22^{phox} mRNA expression	0.65 ± 0.2	1.24 ± 1.08	0.14 ± 0.03	NA	NA
SOD-1 mRNA expression	0.77 ± 0.18	2.20 ± 1.97	0.37 ± 0.14	NA	NA

NA = not available.

Mass indexes were normalized by body weight.

* $P < .05$ v Ang$_L$ and Ang$_L$+GTE; † $P < .001$ v vehicle; ‡ $P < .01$ v Ang$_L$ and Ang$_L$+GTE; § $P < .05$ v vehicle; ‖ $P < .001$ v all other groups; ¶ $P < .001$ v Ang$_H$ and Ang$_H$+GTE.

Data Analysis

Data are expressed as mean ± SEM. The BP and the hydroperoxide changes were analyzed by repeated-measures ANOVA and Mann-Whitney nonparametric tests. Two-way ANOVA was used to compare concentration–response curves between placebo and treated groups and to assess interaction (time × angiotensin dose) of GTE on oxidative stress. Comparison across groups were performed by ANOVA followed by Bonferroni's post-hoc test and Student t test for unpaired observations where appropriate. Probability was set at $P < .05$. The ACh concentration–response data were used to calculate EC$_{50}$ expressed as $-\log$ [M] (pEC$_{50}$) and the maximum relaxation (R$_{max}$).

Results
Daily Water and Green Tea Extract Consumption

There were no differences in food and daily water intake (51.7 ± 5.5 mL) in the study groups. The average GTE intake was 310 ± 3 mg/d.

Effect of Angiotensin II and Green Tea Extract on Blood Pressure and Heart Rate

By day 2 of infusion, the Ang$_H$ group showed significantly higher systolic and diastolic BP during night-time (awake) and daytime (asleep), compared to all others group (Fig. 1). The BP values were also higher in the Ang$_L$ than in the placebo group, but only during the first week of the experiment.

The GTE significantly blunted the increase of BP caused by Ang$_H$. The GTE abolished the pressor effect of Ang$_L$ during the first week of experiment. No effects of Ang$_H$, Ang$_L$, with or without GTE, on heart rate were noticed.

Left and Right Ventricular Remodeling

The LV mass index increased in the Ang$_H$ group ($P < .005$) (Table 1 and Fig. 2). The GTE significantly ($P < .005$) attenuated this increase; however, the LV mass index of the Ang$_H$+GTE group remained higher than in the placebo group ($P < .001$).

No increase of LV mass index was observed in the Ang$_L$ group and in the Ang$_L$+GTE compared to placebo.

No significant changes of RV mass index were observed in all groups compared to placebo (Table 1).

Vascular Remodeling

The media-to-lumen ratio increased ($P < .05$) with Ang$_H$ compared to placebo (Fig. 2, lower panel). The GTE attenuated ($P < .05$) this increase. This index was unaffected by Ang$_L$, alone or with GTE.

Markers of Oxidative Stress and Aortic HO-1, NADPH Oxidase (p22^{phox}), and SOD-1 mRNA

Compared to baseline, the Ang$_H$ group showed an increase of hydroperoxides at the end of the study (Fig. 3); however, the blunting of this increase with GTE did not achieve statistical significance. The Ang$_L$ also increased hydroperoxides after 1 week of the experiment (+47%), but at the end of the study there was a 47% decrease. The increase after 1 week was abolished by co-administration of GTE. An interaction ($P < .05$) of treatment by time was therefore detected by ANOVA (Fig. 3).

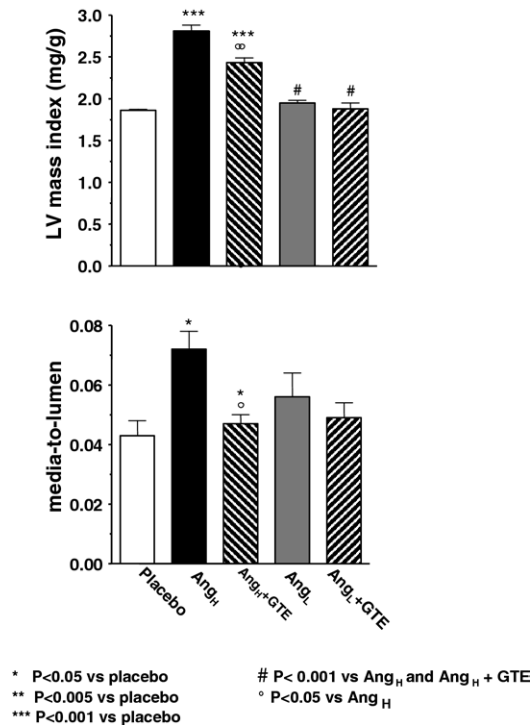Nitrotyrosine levels showed no differences across groups (Table 1).

* P<0.05 vs placebo   # P< 0.001 vs Ang$_H$ and Ang$_H$ + GTE
** P<0.005 vs placebo   ° P<0.05 vs Ang$_H$
*** P<0.001 vs placebo

FIG. 2. The bar graph shows the left ventricular (LV) mass index (**upper panel**) and small mesenteric artery media-to-lumen ratio (**lower panel**) in the various experimental groups. (**Upper panel**) The LV mass index increased on average by about 36% in the Ang$_H$ group ($P <$ .005) by the end of the study. The GTE significantly ($P <$ .005) attenuated this increase. No significant increase of LV mass index was observed in the Ang$_L$ and in the Ang$_L$+GTE group, compared with placebo. (**Lower panel**) A significant ($P <$ .05) increase of the media-to-lumen ratio was observed in the Ang$_H$ group, compared with placebo. The GTE significantly ($P <$ .05) attenuated this increase. In contrast, no significant effects on this index of the Ang$_L$ dose, alone or with GTE, were observed. See Fig. 1 for abbreviations.

The Ang$_H$ markedly increased in HO-1, p22^{phox}, and SOD-1 mRNA in aorta. These effects were abolished by GTE, which reduced the mRNA levels below baseline (Table 1). However, these changes did not attain statistical significance because of the spread of the values.

Endothelial Function

Compared to the placebo, the Ang$_H$ dose determined a significant shift rightward of the concentration–response curve to ACh (Fig. 4, upper panel), indicating a decreased endothelium-dependent relaxation. This was prevented by GTE. The Ang$_L$ did not affect the concentration–response curve to ACh compared with the placebo group (Fig. 4, lower panel); hence, no effect of the in vivo drinking of GTE could be observed. The SNP elicited no significant effect on the concentration–response curve in any group (not shown).

Effect of O$_2^{\cdot-}$ Scavenging

The PE precontracted mesenteric arterioles exposed first to ACh alone, and then to ACh in the presence of tempol were analyzed in a pairwise fashion. Tempol alone did not

appreciably affect the curve observed in the placebo group (not shown). In the Ang$_H$ group, tempol abolished the rightward shift of the curve induced by in vivo administration of Ang II (Fig. 4, upper panel). In contrast, it had no effect on the vessel vasodilatory response to ACh, both in the Ang$_H$+GTE (Fig. 5, upper panel) and Ang$_L$+GTE (Fig. 5, lower panel) groups.

Discussion

The GTE added to drinking water to rats made hypertensive with a 2-week infusion of Ang II prevented the increase of BP and exerted CV protective effects, thus extending findings in other models of hypertension including the CBA mice, the Dahl salt-sensitive rat, the stroke-prone spontaneously hypertensive rat, the 5/6 nephrectomized rat, and the high fructose-fed rat.[5,21,22]

The doses of Ang II previously used were 7000-fold (from 100 ng/kg/d to 700 μg/kg/d).[7,8,15] We administered the highest dose to induce HT and activate NADPH oxidase, thereby enhancing O$_2^{\cdot-}$ generation in the vascula-

* P=0.01 vs baseline;
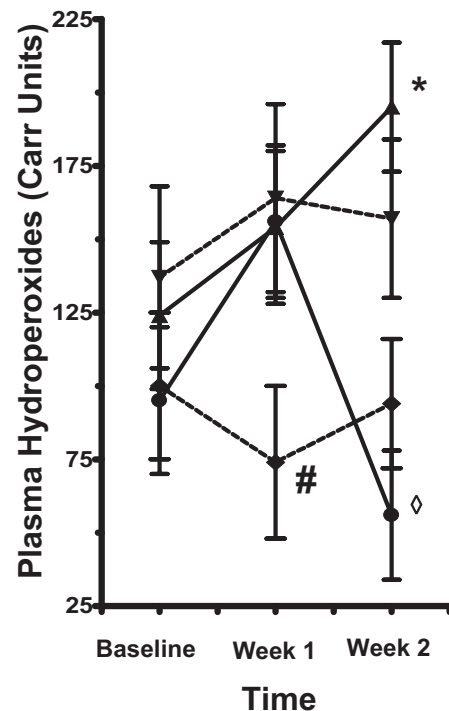# P=0.01 vs Ang$_L$ at week 1;
◊ P<0.05 vs Ang$_L$ at week 1;

FIG. 3. The graph shows the change of hydroperoxides from baseline in the four experimental groups at 1 week and at sacrifice (week 2). The Ang$_H$ (▲) group showed a significant increase of hydroperoxides during the study that was blunted in Ang$_H$ rats receiving GTE (▼). The Ang$_L$ rats (●) showed a clearcut increase of hydroperoxides at week 1, with normalization by the end of the study. In contrast, with Ang$_L$+GTE (♦) the transient increase at week 1 was abolished. By repeated measure ANOVA a significant ($P <$ .05) interaction of treatment × time was detected, due to these divergent changes. See Fig. 1 for abbreviations.
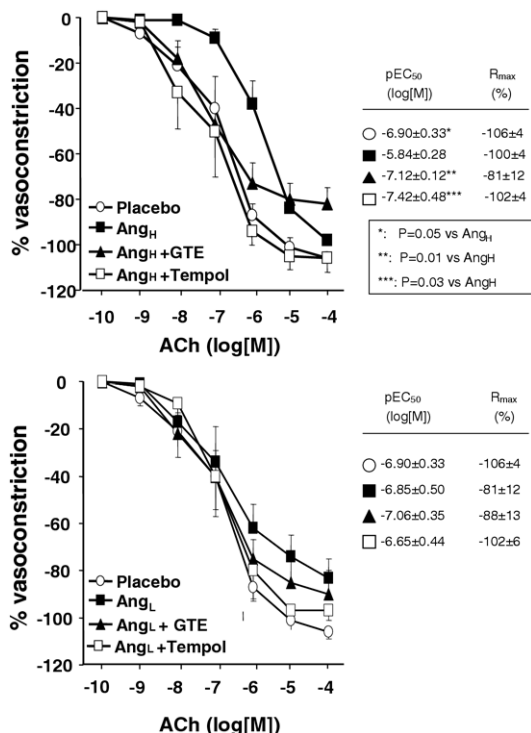
FIG. 4. The graph shows the concentration–response curves of phenylephrine-precontracted small mesenteric arteries to cumulative doses of acetylcholine in the different experimental groups. (**Upper panel**) Ang_H determined a significant ($P = .05$) shift to the right of the concentration–response curve to acetylcholine (ACh) compared with placebo group, indicating a decrease in endothelium-dependent relaxation. This effect was annulled by the co-administration in vivo of GTE and of tempol in vitro. (**Lower panel**) Ang_L did not bring about a significant shift to the right of the concentration–response curve to ACh; no differences were induced by the co-administration of GTE in vivo and tempol in vitro. See Fig. 1 for abbreviations.

ture.[23,24] We also used a lower dose to determine whether it might elicit long-term pressor effects and ED due to its pro-oxidant properties.[13,14,16]

We used telemetry, which has never been used previously in Ang II infusion models of HT for more than 7 days, to measure BP in unrestrained rats to minimize the confounding effects occurring with the tail–cuff technique and restrainer. Interestingly we found that the 700 μg/kg/ Ang II dose increased BP throughout THE study period, whereas the low dose (350 μg/kg/d) elicited a mild pressor effect only during the first week. Why BP returned to normal values afterward in this group is unclear. Changes in sodium intake are unlikely to play a relevant role, as all groups received the identical diet and there were no differences in food intake across groups. More plausible mechanisms entail Ang II receptor subtype 1 downregulation or desensitization, or activation of compensatory vasodilating systems.

Noteworthy, the plasma levels of hydroperoxides (Fig. 3) showed the same biphasic behavior suggesting a close relationship between the hemodynamic and the pro-oxidant effects of Ang_L. Whatever the mechanisms, these results reconcile previous conflicting data on the Ang II

doses that are required to increase BP after 1 and 2 weeks.[10–12]

Effect of Angiotensin II on Cardiovascular Damage

The Ang_H dose induced LV hypertrophy, mesenteric arteriole remodeling (Fig. 2), and ED (Fig. 4), in accordance with previous observations.[9,25,26] No LV hypertrophy was observed at the end of the study with the Ang_L dose (Fig. 2, upper panel), which agrees with the vanished BP-raising effect of the Ang_L dose by the end of week 1 (Fig. 1).

Effect of Green Tea Extract

The GTE attenuated but did not abolish the development of HT with Ang_H (Fig. 1). It remains unclear whether this partial effect depends on the dose of GTE, which might be disproportionately low for the very high Ang II dose used, or on other mechanisms. Noteworthy, GTE prevented LV hypertrophy and vascular remodeling (Fig. 2) and ED (Fig. 4) induced by the Ang_H dose. Thus, GTE exerts BP-lowering and CV protective effects in rats exposed to oxidative stress induced by this very high Ang II dose. Similar effects have been documented in other models with GTE,[22] and other antioxidant nutrients including resveratrol, quercetin,[3] and cocoa.[27]
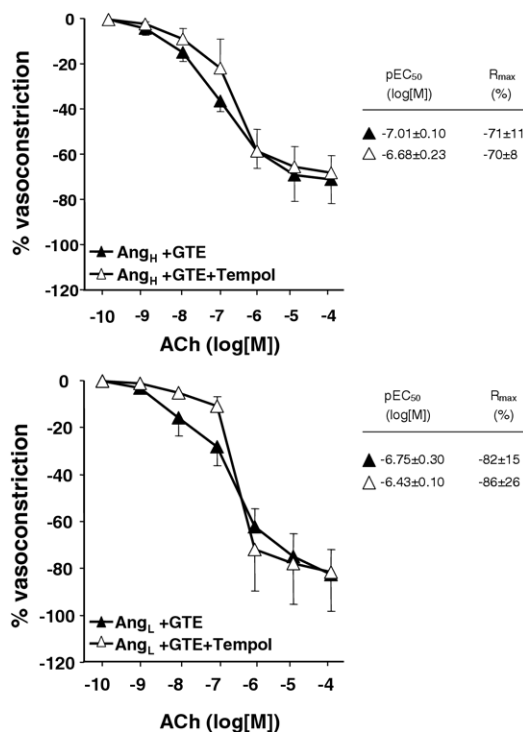

FIG. 5. The graph shows the concentration–response curves of phenylephrine-precontracted small mesenteric arteries to cumulative doses of acetylcholine (ACh) in the rats treated with Ang_H+GTE (**upper panel**) and with Ang_L+GTE (**lower panel**) in the presence ($\triangle$) and absence ($\blacktriangle$) of the superoxide dismutase analog tempol. Tempol did not induce any shift of the concentration–response curve to ACh, suggesting that GTE action involves scavenging of superoxide anion. See Fig. 1 for abbreviations.

The BP-lowering effects of GTE were observed also with the Ang_L dose during week 1, suggesting that GT exerts its CV protective effects over a wide range of Ang II concentrations and prevailing oxidative stress.

The selected dose of GTE exceeds that found in a GT beverage. In addition, the rats were provided continuously with the extract in their drinking water. Based on an average consumption of 1 g/kg of GTE/day the rats received a dose about 70-fold higher than that consumed by a 70-kg human being drinking a liter of GT daily. However, the bioavailability of tea catechin is low in rodents.[28] Furthermore, the amount of drug needed to achieve an effect increases as the size of the animal gets smaller. Therefore, a higher dose of GTE was necessary in rats. In addition, in rats: (1) the EGCG are lower than in mice[13]; (2) the main catechins of GTE (EGCG, epigallocatechin (EGC), and epicatechin (EC)) are largely inactivated, which might not occur to the same extent in humans[14]; and (3) with chronic consumption of GTE there is an "increase-and-then-a-decrease" pattern of levels of catechin plasma concentrations, indicating adaptive responses affecting blood and tissue levels of the tea catechins.[13]

Finally, this high GTE dose was chosen to show a CV protective effect under conditions of exposure to markedly increased Ang II concentrations within the short period of our experiment. Therefore, a lifetime exposure to chronic consumption of GT, as commonly seen in GT drinkers of 10 to 15 cups/day, might also have the striking CV protective effects documented in this study.

Upon completion of this study a large population-based epidemiologic survey in Japan[15] evidenced an inverse relationship between GT consumption and total and CV mortality. Subjects drinking more than 5 cups daily of GT (about 500 mL), but not black or oolong tea, had a 33% reduction in the risk of CV mortality compared to those drinking less than 1 cup during the 11 years of follow-up. This association was seen even when these subjects reported a history of arterial hypertension, indicating that the beneficial effect of GT is peculiar to the hypertensive population. In addition, the association was explained by a reduction of atherothrombotic stroke, a typical complication of hypertension-induced accelerated atherosclerosis. By showing an effect of GTE in lowering BP, preventing cardiac hypertrophy, vascular remodeling, and endothelial dysfunction, possibly by scavenging of oxygen free radicals[22] and ameliorating Ang II-induced hypermeability in endothelial cells,[29] our data offer a mechanistic explanation for those findings. Further research is necessary to establish the long-term BP-lowering effects of a lower GTE dose under less prominent conditions of Ang II-induced hypertension, CV damage, and oxidative stress, and to determine whether there might be a "threshold" effect of GT consumption.[15]

Effect of Angiotensin II and Green Tea Extract

The measurement of plasma hydroperoxides showed that both Ang II doses increased oxidative stress (Fig. 3), although for Ang_L it was only in week 1 when the BP increasing effect was still evident. At comparison, plasma nitrotyrosine levels can be a less sensitive marker for oxidative stress under these experimental conditions, as we found no changes across treatment groups. In relation to baseline, GTE treatment prevented the increase of hydroperoxides seen with Ang_H by the end of 2 weeks (Fig. 3), in agreement with the findings that GTE blunted the increase of lipid peroxides in the aorta and heart seen in diabetic rats.[30] In these animals the induction of antioxidant enzymes (SOD, catalase, and glutathione peroxidase) associated with diabetes, and their ensuing increased activity was not efficient to reduce the oxidative stress. In contrast, GTE markedly ameliorated the oxidative stress, possibly by acting as a free radical scavenger. It also abolished the transient increase observed with the Ang_L dose (Fig. 3), thus showing a significant interaction of Ang_L with GTE.

Because GTE and EGCG did not directly scavenge H_2O_2 and can exert pro-oxidant effects leading to DNA damage in vitro,[8] our results require further investigation. Nonetheless, they are consistent with the following interpretation: (1) enhanced oxidative stress plays a key role in the BP raising and the detrimental CV effects of high-dose Ang II[31]; (2) the CV protective effect of GTE may involve scavenging of $O_2^{\cdot-}$, as observed in the Ang_L group; and (3) the inability of GTE to abolish the pressor effect of Ang_H might partly be because at these doses it did not annul the pro-oxidant action of the high Ang II dose that was used.

The changes in the indexes of oxidative stress were unparalleled by detectable changes of mRNA of HO-1, p22phox, SOD-1—three enzymes involved in regulating the amount of $O_2^{\cdot-}$ in the aortic wall. Thus, the plasma levels of reactive oxygen species might be related to post-transcriptional modulators of enzyme activity, to changes of gene expression in the microcirculation, or sites different from the thoracic aorta.

Effects of $O_2^{\cdot-}$ Scavenging

The Ang_H dose was associated with a blunting of the vasodilatory response to ACh of PE preconstricted resistance arterioles, which was prevented by in vivo treatment with GTE (Fig. 4). The vasodilatory response was restored in vitro by the superoxide scavenger tempol (Fig. 5), which per se had no effect. Thus, the Ang_H dose causes ED by enhancing the generation of $O_2^{\cdot-}$, which is scavenged in vitro by tempol and prevented in vivo by GTE administration.[5,22]

In conclusion, GTE abolished the development of severe LV hypertrophy, vascular remodeling, and ED, induced by chronic infusion of a high dose of Ang II, by lowering of BP and blunting, or scavenging, of $O_2^{\cdot-}$ generation. Because compelling epidemiologic evidence indi-

cates a CV protective effect of GT,[6] further research should explore the potential therapeutic value of GTE in clinical forms of HT with enhanced Ang II generation, such as high-renin essential HT and renovascular HT.

Acknowledgments

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