Black and Green Tea Polyphenols Attenuate Blood Pressure Increases in Stroke-Prone Spontaneously Hypertensive Rats[1]

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ABSTRACT Oxidative stress was reported to be involved not only in cardiovascular diseases, but also in hypertension. Epidemiologic studies indicated that tea consumption slightly reduces blood pressure. We conducted two studies to determine whether black and green tea can lower blood pressure (BP) in stroke-prone spontaneously hypertensive rats (SHRSP). Male SHRSP (n = 15) were allowed to recover for 2 wk after a transmitter for measuring BP was implanted in the peritoneal cavity. The rats were divided into three groups: the control group consumed tap water (30 mL/d); the black tea polyphenol group (BTP) consumed water containing 3.5 g/L thearubigins,0.6 g/L theaflavins, 0.5 g/L flavonols and 0.4 g/L catechins; and the green tea polyphenol group (GTP) consumed water containing 3.5 g/L catechins, 0.5 g/L flavonols and 1 g/L polymetric flavonoids. The telemetry system was used to measure BP, which were recorded continuously every 5 min for 24 h. During the daytime, systolic and diastolic BP were significantly lower in the BTP and GTP groups than in the controls. Protein expressions of catalase and phosphorylated myosin light chain (MLC-p) were measured in the aorta by Western blotting. GTP significantly increased catalase expression, and BTP and GTP significantly decreased MLC-p expression in the aorta. These data demonstrate that both black and green tea polyphenols attenuate blood pressure increases through their antioxidant properties in SHRSP. Furthermore, because the amounts of polyphenols used in this experiment correspond to those in ~1 L of tea, the regular consumption of black and green tea may also provide some protection against hypertension in humans. J. Nutr. 134: 38–42, 2004.

KEY WORDS: • *tea polyphenols* • *hypertension* • *nitric oxide* • *catalase* • *myosin light chain phosphorylation*

Black tea is the most widely consumed beverage worldwide. Three kinds of tea are drunk: black (78%), green (20%) and oolong (2%) (1). Green tea contains many polyphenols known as cathechins, including epigallocathechin-3 gallate, epigallocathechin and epicathechin-3 gallate. In a fermentative process, cut and partially dried green tea leaves are subjected to controlled enzymatic biotransformations at a slightly elevated temperature to give the characteristic color and flavor of black tea. Catechins are the main polyphenolic flavonols of tea that undergo major biotransformations during this operation, leading to the formation of theaflavins and thearubigins, which are the characteristic constituents in black tea (1,2).

Recently, the involvement of reactive oxygen species (ROS)[3] in not only cardiovascular diseases, but also hypertension, was demonstrated in both hypertensive rat models and humans (3–7). Humans maintain defense systems against ROS through enzymes (superoxide dismutase, glutathione peroxidase and catalase) and low-molecular-weight antioxidants. Diets with antioxidant properties include many fruits and vegetables, which are considered to be important sources of antioxidants (8). Dietary antioxidants including vitamin E, vitamin C, carotenoids and polyphenols have received much attention in the prevention of cardiovascular disease and its risk factors (8).

Tea polyphenols, catechins and flavonols scavenge ROS (9) and chelate transition metal ions in a structure-dependent manner (10). Flavonoids found in tea scavenge nitric oxide (NO) and peroxynitrite produced from superoxide radicals and NO (11,12), effectively reducing the bioavailability of endothelium-derived NO. It was shown that any possible production of peroxynitrite could be eliminated by black tea or its characteristic constituent theaflavins by simply preventing the induction of inducible NO synthase synthesis (2). Furthermore, epidemiologic studies suggested that tea polyphenols that can be derived from black and green tea may protect against cardiovascular diseases (13–17). Therefore, the physi-

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[3] Abbreviations used: BP, blood pressure; BTP, black tea polyphenols; DBP, diastolic blood pressure; GTP, green tea polyphenols; MLC, myosin light chain; MLC-p, phosphorylated myosin light chain; NOS, nitric oxide synthase; ROS, reactive oxygen species; SBP, systolic blood pressure; SHRSP, stroke-prone spontaneously hypertensive rats.

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ologic effects of tea and its components on cardiovascular disease risk factors such as hypertension are of interest.

In this study, the protective effects of black and green tea polyphenols on hypertension were examined. In particular, the effect of black and green tea polyphenols, which have antioxidant effects, was assessed on the contraction/relaxation of aorta in SHRSP.

MATERIALS AND METHODS

Study 1

Animals. Male stroke-prone spontaneously hypertensive rats (SHRSP)/Izm ($n = 15$) at 13 wk of age were divided into three groups of 5; the control group consumed tap water, the black tea polyphenols group (BTP) consumed water containing 3.5 mg/L thearubigins, 0.6 g/L theaflavins, 0.5 g/L flavonols and 0.4 g/L catechins and the green tea polyphenols group (GTP) consumed water containing 3.5 mg/L catechins, 0.5 g/L flavonols and 1 g/L polymetric flavonoids. Water (30 mL/d) containing 150 mg tea polyphenols was given to each of the rats. All groups consumed a nonpurified laboratory diet (Funabashi Farm, Chiba, Japan) ad libitum. All rats were housed one per cage. Body weight and blood pressure (BP) were determined before allocation to groups to ensure weight and BP homogeneity. The environment was controlled at 23 ± 1°C with a 12-h light:dark cycle. After the rats were anesthetized with pentobarbital sodium at 17 wk of age, the blood and heart were removed, and the heart weight was determined.

Telemetry transmitter implant surgery. Telemetry probes of an implantable device [TA11PA-CA40, Data Sciences International (DSI), St. Paul, MN] were implanted when the rats were 11 wk of age and their body weights were between 218 and 262 g. After the rats were anesthetized with Nembutal (pentobarbital sodium), a pressure catheter, which contains a biocompatible gel at the tip and a noncompressible liquid connecting the tip to the pressure sensor, was implanted though the femoral artery until it reached all the way up the abdominal aorta. A small puncture hole was sealed in place with 3M Vetbond tissue adhesive (DSI) and a cellulose fiber patch (DSI). The telemetry device body, which includes the pressure sensor, the electronics module and battery, was fixed to the muscle wall and was left in the abdominal cavity. After the surgery, the rats were allowed to recuperate for at least 10 d.

Blood pressure measurements. Rats were placed individually in cages on telemetry receivers. The Physiotel telemetry system (Dataquest IV, DSI) comprised telemetry implant pressure transmitters (TA11PA-CA40), data receivers (RLA 1010), an ambient pressure reference monitor, a DCM100 consolidation matrix and a DATAquest system computer, including Dataquest LabPro data acquisition software (18). Each telemetry probe was calibrated before and cleaned after each use according to the manufacturer's instructions.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were monitored continuously from conscious, unrestrained rats at a sampling rate of every 5 min. Measurements ($n = 288$) were obtained for each rat over 24 h for each variable; the mean values during the daytime (resting time: 0600–1759 h), and during the nighttime (activity time: 1800–0559 h) were used for analysis.

Plasma catechin analysis. EDTA blood was centrifuged at 1700 × *g* for 10 min at room temperature. The plasma was collected and 100 μL of ascorbic acid-EDTA solution was added to 900 μL plasma. This solution contained 0.4 mol/L NaH_2PO_4, 1.14 mol/L ascorbic acid and 2.7 mmol/L EDTA-2Na adjusted to pH 3.6 with 10 mol/L NaOH. After the addition of the ascorbic acid-EDTA solution, the pH of the plasma samples was ~5.0. All samples were frozen under nitrogen gas at −80°C. Frozen plasma samples were transported to Unilever Research Vlaardingen, Netherlands by air, and analyzed for tea polyphenols by HPLC, using the method of Lee et al. (19). The samples were incubated with a mixture of β-glucuronidase and sulfatase to generate the free form of tea polyphenols. After extraction into ethyl acetate and separation by reversed-phase HPLC, epigallocathechin gallate, epigallocathechin and epicathechin were identified by their retention times and electrochemical characteristics.

Study 2

Animals. Male SHRSP/Izm ($n = 15$) at 13 wk of age were divided into three groups of 5, i.e., the controls, BTP and GTP. All rats were housed as in Study 1. Urine was collected for 24 h from rats housed in metabolic cages (NALGENE, Nalge, New York, NY) at 16 wk of age. The volume of urine collected was recorded. One day after the 24-h urine collection, all rats were anesthetized with pentobarbital sodium. The blood and aorta were removed immediately. The tissues were rapidly frozen in liquid nitrogen and stored at −80°C until processing.

Urinary and plasma nitric oxide concentrations were measured by the Griess method [NO_2/NO_3 Assay kit-C (Colorimetric) Dojindo, Kumamoto, Japan]. The range of the standard curve was from 0 to 100 μmol/L. Plasma samples were assayed after ultrafiltering using centrifugal filter devices (CENTRICON YM-10, Millipore, Bedford, MA). Both urine and plasma samples were read at 560 nm in a 96-well Spectra Microplate Autoreader (Tecan Spectra Classic, Tecan Austria, Groedig, Austria).

Tissue preparation and Western blot analyses. Proteins were extracted in boiling 0.5 mmol/L Tris/HCl, pH 6.8, glycerol, 10% SDS, 0.1% bromophenol blue and 2-mercaptoethanol. Protein was electrophoresed on a stacking gel. The proteins were transferred onto a nitrocellulose membrane for 2 h. The membrane was blocked in 5% skimmed milk in washing buffer (TBS-Tween) overnight. After appropriate blocking, the blot was incubated with anti-catalase rabbit polyclonal antibody (1:1000; Abcam Limited, Cambridge, UK), anti-myosin light chain (MLC) (p-18) and -MLC phosphorylation (Thr18/ Ser19) goat polyclonal antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h. It was then washed and finally incubated for 1 h with 1:1000 dilution of anti-goat IgG antibody (Santa Cruz Biotechnology) or anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Piscataway, NJ) in washing buffer. Optical densities were measured using NIH Image Software (v. 1.62).

Statistics. Results are presented as means ± SEM. Differences between the control group and the experimental groups were tested using Dunnett's test. Statistical comparisons between BP during daytime and nighttime were done by Student's paired *t* test. Probability values < 0.05 were considered significant.

RESULTS

Study 1: blood pressure and plasma catechins. The mean daytime SBP was significantly lower in the BTP and GTP groups than in the control group on d 15, and in the GTP group on d 18 (**Fig. 1***A*). The mean daytime DBP was significantly lower in the BTP group than in the controls on d 13, 15, 16 and 18 and on d 15 and 18 in the GTP group (Fig. 1*B*). DBP during the nighttime was significantly lower in the BTP group than in the controls on d 14 (**Fig. 2***B*). However, at the end of this study, significant differences in BP could not be detected because the within-group variation was large in the controls.

In the BTP and GTP groups, BP was greater during the night than during the day (normal BP pattern). In controls, daytime and nighttime BP did not differ on d 15 (abnormal diurnal variation pattern; nondipping), and daytime BP was higher than nighttime BP on d 18 (abnormal diurnal variation pattern; reverse dipping) (Fig. 2). Plasma total catechins were higher in the GTP group than in the controls (**Fig. 3**).

Study 2: tea polyphenol antioxidant activity. The plasma concentration of NO was higher in controls than in the BTP and GTP groups (**Fig. 4***A*), and urinary NO excretion was higher in controls than in the BTP group (Fig. 4*B*). Aorta MLC phosphorylated protein (MLC-p) expression was lower in rats fed BTP and GTP than in the controls (**Fig. 5**). Aorta catalase protein expression was greater in the GTP group than in controls (**Fig. 6**).

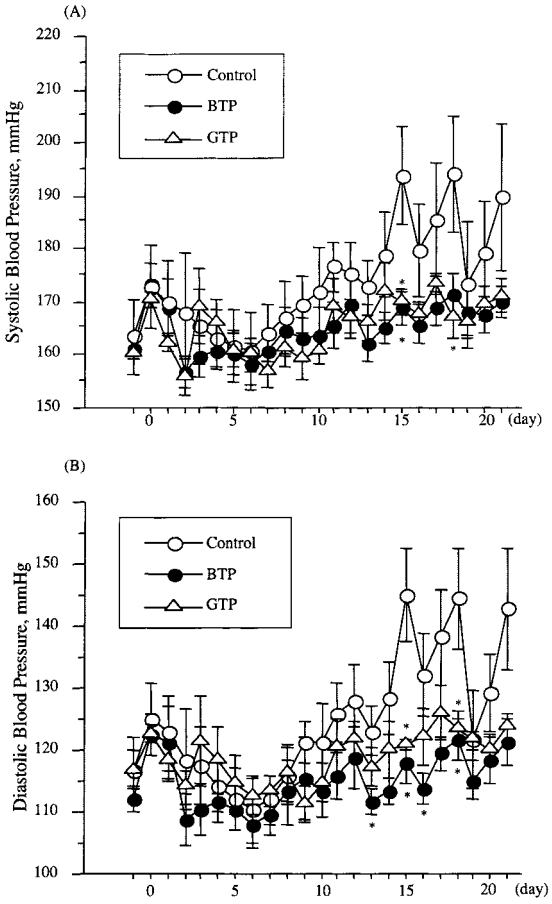

FIGURE 1 Daytime systolic (*A*) and diastolic (*B*) blood pressure of stroke-prone spontaneously hypertensive rats (SHRSP) that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± *SEM, n* = 5. *Different from the control, P < 0.05.

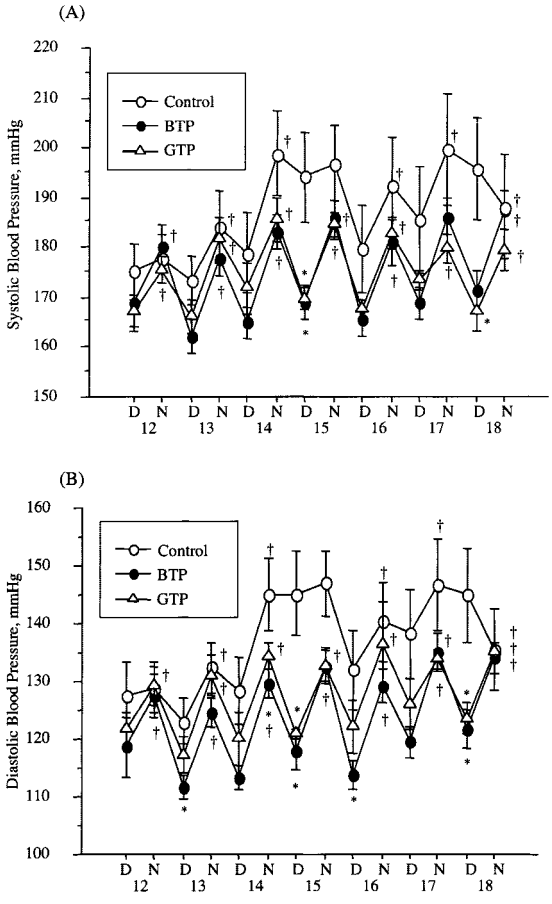

FIGURE 2 Systolic (*A*) and diastolic (*B*) blood pressure during daytime (D) and nighttime (N) from d 12 to 18 of stroke-prone spontaneously hypertensive rats (SHRSP) that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± SEM, *n* = 5. *Different from the control, *P* < 0.05. †Different from the corresponding daytime BP, *P* < 0.05.

DISCUSSION

Tea polyphenols attenuated the development of hypertension in SHRSP. Several possibilities are responsible for the reduction on hypertension in SHRSP. First, McIntyre et al. (20) examined the role of the superoxide anion ($O_2^{\cdot-}$) in relation to endothelial dysfunction. NO can be scavenged by $O_2^{\cdot-}$ to form peroxynitrite, effectively reducing the bioavailability of endothelium-derived NO. Recent reports suggested that NO has a negative-feedback role in the regulation of endothelial NOS expression (21), and increased ROS-mediated inactivation of NO can potentially contribute to a compensatory upregulation of nitric oxide synthase (NOS) via a reduction in NO availability in lead-induced hypertension (22). In addition, potent antioxidant therapy for ameliorating hypertension lowered urinary NO metabolite excretion and reduced the compensatory upregulation of NOS isotypes in vascular, renal and cardiac tissue (23). Kerr et al. (24) reported that the increase in $O_2^{\cdot-}$ generation in SHRSP could contribute to the decreased availability of basal NO observed in this model of genetic hypertension.

In the present study, the controls exhibited an elevation in blood pressure and higher plasma NO concentrations than that in either tea polyphenol group; urinary NO metabolic excretion was higher in controls than in the BTP group. The intake of tea polyphenols for 3 wk attenuated BP increases despite a marked reduction in plasma NO concentration and urinary NO excretion. These data suggest that alleviation of oxidative stress by tea polyphenols diminishes ROS-mediated

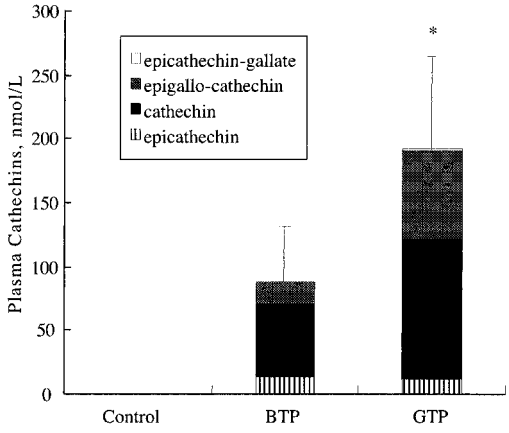

FIGURE 3 Plasma catechin concentrations at 17 wk of age in stroke-prone spontaneously hypertensive rats (SHRSP) that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± SEM, *n* = 5. *Different from the control, *P* < 0.05.

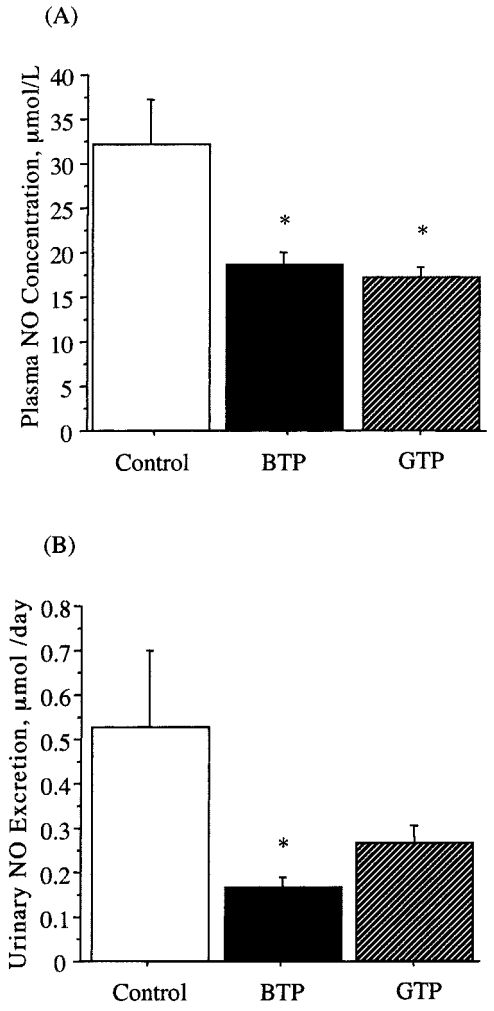

FIGURE 4 Plasma NO concentration (*A*) and urinary NO excretion (*B*) at 16 wk of age in stroke-prone spontaneously hypertensive rats (SHRSP) that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± SEM, $n = 5$. *Different from the control, $P < 0.05$.

NO inactivation and raises the bioavailability of NO in the black and green tea polyphenol groups. The rise in the NO bioavailability enhances NO-mediated vasodilatory tone, which could account for the observed amelioration of hypertension.

Second, the major regulatory mechanism of smooth muscle contraction is phosphorylation/dephosphorylation of the MLC. MLC is phosphorylated by the Ca^{2+}-calmodulin-activated MLC kinase and dephosphorylated by the Ca^{2+}-independent MLC phosphatase (25). Kureishi et al. (26) demonstrated that Rho-kinase directly modulates smooth muscle contraction through MLC phosphorylation, independently of the Ca^{2+}-calmodulin-dependent MLC kinase pathway. In addition, ROS could increase the phosphorylation of MLC by activating the MLC kinase and/or by inhibiting the MLC phosphatase (27). RhoA stimulates a variety of downstream targets, including Rho-kinase and serine/threonine kinase; Rho-kinase was shown to phosphorylate the myosin-binding subunit of MLC phosphatase, leading to the inhibition of phosphatase activity (28). Chitaley et al. (29) reported that decreased NO bioavailability leads to an increase in RhoA/Rho-kinase contractor activity. In this study, we observed decreased phosphorylation of MLC in the aorta of rats fed

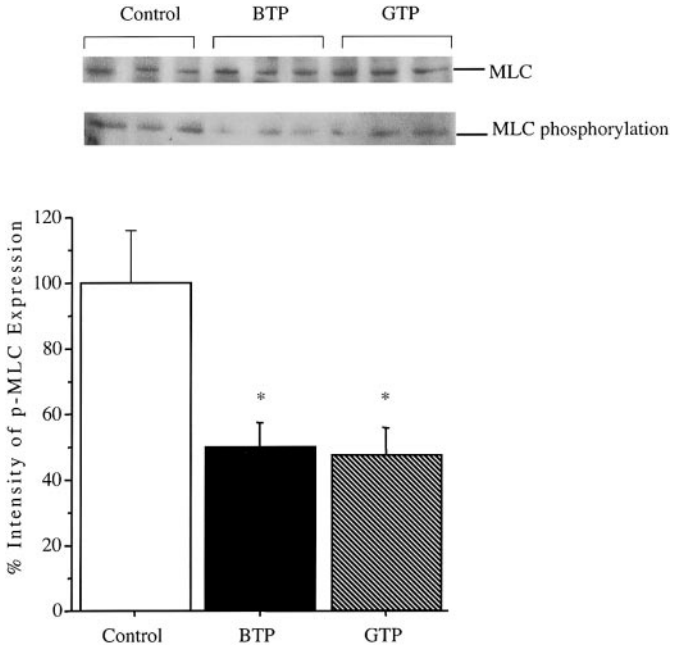

FIGURE 5 Myosin light chain (MLC)-phosphorylated protein expression (Thr18/Ser19) by Western blotting in the aorta of stroke-prone spontaneously hypertensive rats (SHRSP) at 16 wk of age that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± SEM, $n = 3$. *Different from the control, $P < 0.05$.

black or green tea polyphenols. This finding suggests that the inhibition of MLC phosphorylation contributes to the attenuation of blood pressure increases. It was postulated that the phosphorylation of MLC may be reduced by the inhibition of Rho-kinase constrictor activity through the increase in NO bioavailability in the treated SHRSP with tea polyphenols (20,23,24,27–29).

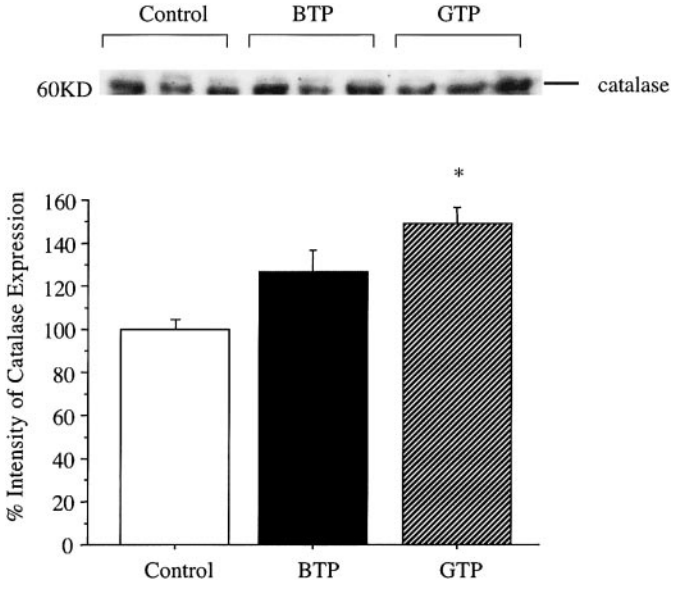

FIGURE 6 Catalase protein expression by Western blotting in the aorta of stroke-prone spontaneously hypertensive rats (SHRSP) at 16 wk of age that consumed water (control), or water containing black tea polyphenols (BTP) or green tea polyphenols (GTP) for 3 wk. Values are means ± SEM, $n = 3$. *Different from the control, $P < 0.05$.

Third, the only ROS scavenger found to completely abolish the contractile response is catalase, a specific scavenger of H_2O_2. It has been reported that H_2O_2 induces Ca^{2+}- and MLC phosphorylation-independent contraction in pulmonary and systemic arterial and venous smooth muscle (30). Our previous study showed that both BTP and GTP groups upregulate catalase level in bovine carotid artery endothelial cells (31). In this study, treatment with GTP significantly increased catalase expression in rat aorta. These findings may support the hypothesis that tea polyphenols inhibit smooth muscle contraction to regulate the endothelial ROS levels though the upregulation of catalase.

In addition, we observed reverse-dipping of BP in the controls, and BP decreases during daytime, which is the normal BP pattern, in the BTP and GTP groups. In humans, BP decreases during sleep by 10–20% and increases promptly on waking (32). In some hypertensive patients, however, a variety of abnormal diurnal variation patterns were described in which the nocturnal fall in BP may be <10% (nondippers), or even reversed (reverse-dippers) (32).

Some studies reported that the nondipping or reverse-dipping pattern of nocturnal BP was an independent predictor for cardiovascular disease (33,34). Hypertensive subjects with the reverse-dipping pattern had a higher incidence of strokes than those with the nondipping pattern (32). These findings suggest that the nondipping or reverse-dipping patterns of BP, which are closely associated with cardiovascular diseases, may be improved by the intake of tea polyphenols.

In conclusion, our findings suggest that black and green tea polyphenols attenuated the development of hypertension through their antioxidant properties in SHRSP because we observed decreased MLC phosphorylation related to NO bioavailability and an increase in catalase, a scavenger of H_2O_2 in rat aorta. Furthermore, because the amounts of polyphenols used in this experiment correspond to those in ~1 L of tea, the regular consumption of black and green tea may also offer some protection against hypertension in humans.

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