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A reduction of endogenous asymmetric dimethylarginine contributes to the effect of captopril on endothelial dysfunction induced by homocysteine in rats

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Abstract

We examined whether captopril exerts beneficial effect on homocysteine-induced endothelial dysfunction in vivo and whether this effect of captopril is associated with a reduction of endogenous inhibitor of nitric oxide synthase (NOS) asymmetric dimethylarginine (ADMA) in rats. Male Sprague–Dawley rats were given intravenous injections of homocysteine (10 mg/kg/day) to induce endothelial dysfunction. Captopril treatment (3 mg/kg/day, i.v.) was taken in some rats after homocysteine administration. Endothelium-dependent relaxation was tested in aortic rings. Serum levels of ADMA, nitrite/nitrate, malondialdehyde (MDA), and creatinine were measured. Furthermore, superoxide dismutase activity in liver and angiotensin converting enzyme activity in serum were also assayed. Administration of homocysteine to rats for 4 weeks significantly impaired endothelium-dependent relaxation compared with control rats. This impairment of endothelium-dependent relaxation was accompanied by elevated serum concentration of ADMA and decreased serum content of nitrite/nitrate. Moreover, serum concentration of MDA was remarkably increased, whereas liver superoxide dismutase activity was decreased in homocysteine-treated group compared with control. Chronic captopril treatment not only improved the impaired endothelium-dependent relaxation, but also prevented the elevation of serum ADMA and MDA levels, as well as reduction of serum nitrite/nitrate contents and liver superoxide dismutase activity. Serum angiotensin converting enzyme activity and creatinine had no significant difference between the three groups. These results suggest that chronic captopril treatment reduces endogenous inhibitor of NOS in rats with homocysteine injection, which may contribute to the beneficial effect of captopril on homocysteine-induced endothelial dysfunction in vivo, and may be secondary to the antioxidative action of captopril.

Keywords: Captopril; Homocysteine; Endothelium-dependent relaxation; ADMA (Asymmetric dimethylarginine); NO (Nitric oxide); (Rat)

1. Introduction

Homocysteine, an intermediate in the metabolism of methionine, has been considered as an independent risk factor for atherosclerosis. It is well known that endothelial dysfunction plays a crucial role in the pathogenesis of vascular diseases such as atherosclerosis, hypertension, and diabetic vascular complications. Endothelial dysfunction is characterized by impaired function of the endothelium, associated with decreased production of nitric oxide (NO) and reduced endothelium-dependent vasorelaxation in response to acetylcholine. A great deal of studies have demonstrated that the function of endothelium-dependent relaxation is impaired in humans and animals with hyperhomocysteinemia (Böger et al., 2000, 2001; Stühlinger et al., 2003; Sydow et al., 2003). Our recent study has showed that exposure normal arteries of rats to homocysteine in vitro significantly inhibited endothelium-dependent relaxation (Fu et al., 2003). However, the mechanisms for homocysteine impairing endothelium-dependent relaxation are not fully understood. Recently, several studies have

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revealed that methylated arginine compounds such as asymmetric dimethylarginine (ADMA) is remarkably raised in hyperhomocysteinemic humans and animals whose endothelium-dependent relaxation of arteries was impaired (Böger et al., 2000, 2001; Stühlinger et al., 2003; Sydow et al., 2003). These results suggest that elevated endogenous ADMA may be a contributor to the homocysteine-induced endothelial dysfunction.

ADMA, an arginine analogue, is an endogenous competitive inhibitor of nitric oxide synthase (NOS), which decreases the production of NO in endothelial cells, subsequently impairing the NO-mediated and endothelium-dependent vasodilatation (Vallance et al., 1992a). ADMA is thought to be continuously produced in the process of metabolism from the proteins containing methylated arginine residues and released as the proteins are hydrolyzed (Najbauer et al., 1993). A number of cells elaborate ADMA, including vascular endothelial cells (Fickling et al., 1993). ADMA is excreted in urine and metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to L-citrulline and dimethylamine (Vallance et al., 1992b; MacAllister et al., 1994). Elevated serum ADMA levels have been shown to be associated with impaired endothelium-dependent vasodilatation in animals and individuals with atherosclerosis, hyperhomocysteinemia, hypertension, diabetes, and in aging (Yu et al., 1994; Böger et al., 2000, 2001; Stühlinger et al., 2003; Sydow et al., 2003; Delles et al., 2002; Xiong et al., 1996, 1997, 2001, 2002, 2003). Thus, elevated endogenous ADMA is an independent risk factor for endothelial dysfunction. Although several studies show that insulin or metformin, and angiotensin converting enzyme inhibitors or angiotensin II type I receptor blocker can reduce plasma ADMA levels in diabetics and hypertensive patients (Asagami et al., 2002; Delles et al., 2002; Ito et al., 2002; Xiong et al., 2003; Napoli et al., 2004), there is no effective pharmacological approach in preventing the elevation of endogenous ADMA levels in hyperhomocysteinemia.

Captopril is an angiotensin converting enzyme inhibitor and is widely used in the treatment of cardiovascular diseases. Captopril has been proven effective in treating hypertension, inhibiting the progression of atherosclerosis, and decreasing mortality of congestive heart failure. Furthermore, captopril has been shown to improve endothelial function in atherosclerosis and hypertension (Keaton et al., 1998; Mancini, 1996). Recently, we have found that captopril can restore endothelium-dependent relaxation of rat aortic rings after exposure to homocysteine (Fu et al., 2003). However, the mechanisms underlying the beneficial effect of captopril on endothelial dysfunction induced by homocysteine have not been elucidated. According to the key role of ADMA in endothelial dysfunction and the beneficial effects of captopril on homocysteine-induced endothelial dysfunction, it is important to determine whether captopril reduces endogenous ADMA levels to improve endothelial dysfunction induced by homocysteine. Therefore, we have undertaken the present study to investigate the effects of chronic treatment with captopril on endothelium-dependent vasodilator function of aorta, serum ADMA levels, and serum angiotensin converting enzyme activity in rats with intravenous injection of homocysteine for 4 weeks.

2. Materials and methods

2.1. Experimental protocol

The Animal Care and Use Committee of Central South University approved the study protocol. Male Sprague–Dawley rats (weighing 200–220 g) were obtained from the Central South University Animal Services (Changsha, PR China). After a 1-week adaptive period, rats were randomly divided into age- and weight-matched control, homocysteine, and homocysteine plus captopril groups. Some rats were given tail vein injections with homocysteine (10 mg/kg/day, dissolved in phosphate buffer solution, pH 7.4) once a day for 4 weeks as homocysteine-treated group. Other rats were given tail vein injections with homocysteine followed by immediate infusions of captopril (3 mg/kg/day, dissolved in phosphate buffer solution, pH 7.4) for 4 weeks as captopril-treated group. Another group of rats was administered with equal volume of phosphate buffer solution for 4 weeks and used as age-matched control group. Animals were placed at cages with free access to food and water in an air-conditioned room under a 12-h light (7 AM to 7 PM), 12-h dark (7 PM to 7 AM) cycle.

2.2. Vascular function assay

At the end of the 4-week period, rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). Blood samples were collected from the carotid artery for biochemical assay. The thoracic aorta was immediately isolated and placed in 4 °C Krebs bicarbonate buffer containing (in millimole) NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.0; and then cleaned of residual blood, fat, and loose connective tissue. The aortic segment was cut into rings of 3–4 mm in length, taking special care to avoid stretching and touching the luminal surface. The rings were suspended horizontally between 2 stirrups in organ chambers filled with 5 ml Krebs' solution at 37 °C and aerated continuously with 95% O_2 and 5% CO_2. One stirrup was connected to an anchor, and the other was connected to a force transducer for recording isometric tension. The solution in the chambers was changed every 15–20 min. Rings were equilibrated for 60 min under 2 g resting tension, and then challenged with 60 mM KCl for at least 3 times until a reproducible maximal contractile response was obtained. After repeated washing and a further equilibration for 30 min, the rings were contracted with a submaximal

concentration of phenylephrine (1 μM), and relaxation response to cumulative concentrations of acetylcholine (0.03–3 μM) was tested at the plateau phase of phenylephrine contraction. Before finishing the experiment, relaxation with sodium nitroprusside (10 μM) at the plateau phase of phenylephrine contraction was also tested.

2.3. Biochemical assays

Blood samples were kept in ice-bath for 2 h and then centrifuged at 1000 ×*g* for 15 min (4 °C). Serum was aliquoted and stored at −70 °C until assayed for ADMA, nitrite/nitrate, malondialdehyde (MDA), angiotensin converting enzyme activity, and creatinine.

2.3.1. Measurement of ADMA and nitrite/nitrate content

Serum (1.0 ml) was pipetted into a tube containing 5-sulpfosalicylic acid (20 mg), and the mixture was stored at 4 °C for 10 min. The precipitated protein was removed by centrifugation at 2500 ×*g* for 15 min (4 °C), and the supernatant was used for the measurement of ADMA and SDMA by high-performance liquid chromatography (HPLC) method as described previously (Chen et al., 1997; Xiong et al., 2001, 2003). The mean recovery of ADMA was over 96% and the inter- and intra-assay coefficients of variation of amounts were lower than 3.80% and those of retention time were below 0.37% for five runs. The detection limit of the assay is 1 pmol when the signal-to-noise ratio is 3:1. We can differentiate ADMA and SDMA in this method.

The serum level of NO was determined indirectly as the content of nitrite and nitrate. Plasma level of nitrite and nitrate was measured as previously described (Jiang et al., 2002). Briefly, nitrate was converted to nitrite with aspergillus nitrite reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 550 nm using a spectrophotometer.

2.3.2. Assays for serum MDA levels and hepatic superoxide dismutase activity

The serum concentration of thiobarbituric acid reactive substance, reflecting the level of lipid peroxidation, was measured using a spectrofluorometer, as described by Yagi (Yagi, 1976), and expressed as the amount of MDA.

After isolating the thoracic aorta, the right lobe of liver was quickly excised and frozen in liquid nitrogen and stored at −70 °C until assay for superoxide dismutase activity. A part of the liver tissue was cut off and homogenized in ice-cold saline. The homogenates were centrifuged at 3000 ×*g* for 15 min (4 °C), and the supernatants were assayed for superoxide dismutase activity and protein content. The activity of SOD was determined by monitoring the inhibition of the autoxidation of hydroxylamine by previously described methods (Kono, 1978), and the protein of the supernatant was detected by Coomassie brilliant blue method.

2.3.3. Assays for angiotensin converting enzyme activity and creatinine level in serum

The activity of serum angiotensin converting enzyme was measured by hydrolysis of hippuril–glycyl–glycine. Briefly, serum (150 μl) was pipetted into a tube containing 14 μM hippuril–glycyl–glycine (100 μl), and then the mixture was incubated at 37 °C for 60 min. The conversion of the substrate was stopped by adding 250 μl of 1 N HCl, after which 1.5 ml acetate ethyl was also added in order to extract the hippuric acid product. The samples were then centrifuged at 1250 ×*g* for 15 min (4 °C). A 500 μl aliquot of the supernatant was pipetted into another tube and then was evaporated at 120–130 °C for 5 min to remove acetate ethyl. Subsequently, 3.0 ml of 1 M NaCl was added to the tube, and absorbance was determined at 228 nm using a spectrometer. We defined the unit of ACE activity as the amount of hippuric acid (μM) per min cleaved by enzyme. In addition, serum creatinine was determined using a routine method.

2.4. Reagents

All chemicals were of the highest purity available. Captopril, D,L-homocysteine, phenylephrine, acetylcholine, sodium nitroprusside, and ADMA were purchased from Sigma (St Louis, MO, USA). Thiobarbituric acid was obtained from Fluka (Milwaukee, WI, USA). Superoxide dismutase, Coomassie brilliant blue, and nitrite/nitrate assay kits were from Nanjing Jiancheng Bioengineering institute (Nanjing, PR China). The commercial kits of serum angiotensin converting enzyme and creatinine were obtained from Navy General Hospital (Beijing, PR China) and Zhongsheng Bioengineering (Beijing, PR China), respectively.

2.5. Statistical analysis

Relaxation was calculated and expressed as percentage of contraction elicited by phenylephrine. The half maximum effective concentration (EC_{50}) response to acetylcholine was estimated by linear regression from log concentration–effect curves. Results are expressed as mean±S.E. The significance of differences between groups was tested with one-way analysis of variance followed by Newman–Keuls test. $P<0.05$ was considered significant.

3. Results

3.1. Vascular function

The contractile responses to phenylephrine in aortic rings from 3 groups were not significantly different (0.97±0.07 g, 1.11±0.09 g, and 1.13±0.05 g for control, homocysteine, and homocysteine plus captopril group, respectively; $n=6$; all P=NS). When the phenylephrine-induced contraction has reached a plateau, acetylcholine (0.03–3 μM) evoked a concentration-dependent relaxation in rings from control

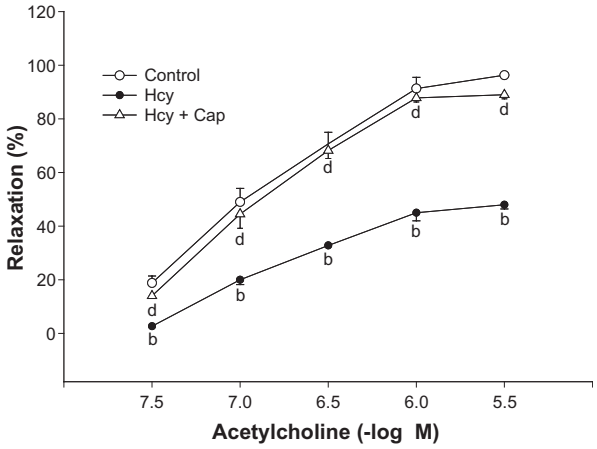

Fig. 1. Effect of captopril treatment on impaired endothelium-dependent relaxation of aortas induced by homocysteine in vivo. Thoracic aortic rings from control, homocysteine (Hcy), and homocysteine plus captopril (Hcy+Cap) group rats were contracted with a submaximal concentration of phenylephrine (1 μM) and the relaxation response to cumulative concentration acetylcholine was determined. Relaxation was calculated and express as percentage of contraction elicited by phenylephrine. Each data point represents the mean±S.E. of results from 6 rats. [b]$P<0.01$ vs control group; [d]$P<0.01$ vs homocysteine group (Newman–Keuls test).

group. The maximal response (E_{max}) reached 96.3±1.2% and the EC_{50} value was 109.1±17.4 nM. In aortic rings from homocysteine-treated group, vasodilator responses to acetylcholine were significantly inhibited, as shown by the lower E_{max} and the higher EC50 value compared with control group. Captopril administration (3 mg/kg/day for 4 weeks) significantly attenuated the homocysteine-induced inhibition of vasodilator response to acetylcholine. The E_{max} and EC_{50} value returned to the levels similar to control group (Fig. 1 and Table 1). By contrast, the endothelium-independent relaxation caused by sodium nitroprusside (10 μM) in aortic rings was not significantly different among the 3 groups (data not shown).

3.2. Serum levels of ADMA and nitrite/nitrate

Vein injections of homocysteine (10 mg/kg/day) to rats for 4 weeks significantly increased serum levels of ADMA,

Table 1
Effects of homocysteine (Hcy) and captopril (Cap) treatment on the E_{max} and EC_{50} values for acetylcholine-induced relaxation in rat aortic rings

Group	n	E_{max} (%)	Acetylcholine	95% CI
			EC_{50} (nM)	(nM)
Control	6	96.3±1.2	109.1±17.4	60.8–157.4
Hcy	6	48.0±1.6[b]	190.0±17.1[b]	142.6–237.5
Cap+Hcy	6	89.0±1.5[d]	107.0±17.9[d]	57.3–156.7

The maximal relaxation (E_{max}) to 3 μM acetylcholine is expressed as percentage of contraction elicited by phenylephrine (1 μM). The half maximum effective concentration (EC_{50}) response to acetylcholine was calculated by linear regression from log concentration–effect curves of acetylcholine. Data are the mean±S.E. of results from n rats.
[b] $P<0.01$ vs control group.
[d] $P<0.01$ vs Hcy group (Newman–Keuls test).

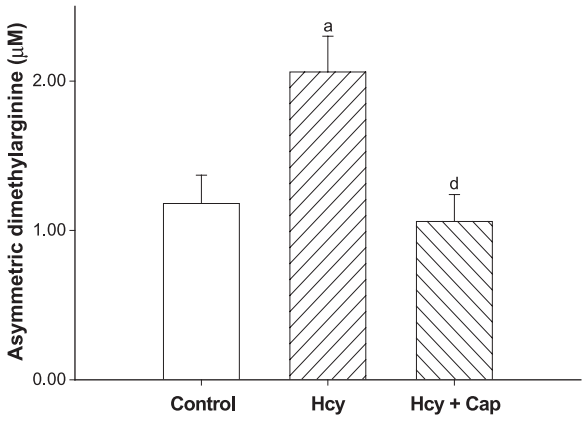

Fig. 2. Effect of captopril treatment on serum asymmetric dimethylarginine levels in rats with homocysteine intravenous injection. Concentrations of ADMA in serum from control, homocysteine (Hcy), and homocysteine plus captopril (Hcy+Cap) group rats were measured by high-performance liquid chromatography. Data are expressed as mean±S.E., n=6. [a]$P<0.05$ vs control group; [d]$P<0.01$ vs Hcy group (Newman–Keuls test).

an endogenous inhibitor of NOS, and decreased serum concentrations of nitrite/nitrate, the stable end products of NO, when compared with control rats (Figs. 2 and 3). Treatment with captopril (3 mg/kg/day for 4 weeks) not only prevented the elevation of serum ADMA concentrations induced by homocysteine (Fig. 2), but also attenuated the reduction of serum nitrite/nitrate levels by homocysteine (Fig. 3). However, serum SDMA concentrations were not significantly different between control, homocysteine, and homocysteine plus captopril group (data not shown).

3.3. Serum MDA levels and hepatic superoxide dismutase activity

Serum concentrations of MDA, derived from lipid peroxidation, were remarkably increased, while the activity

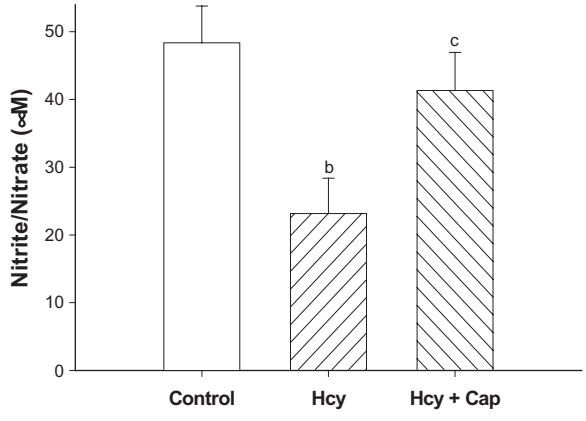

Fig. 3. Effect of captopril treatment on serum nitrite/nitrate contents in rats with homocysteine intravenous injection. Contents of total nitrite in serum from control, homocysteine (Hcy), and homocysteine plus captopril (Hcy+Cap) group rats were determined with the Griess reagent. Data are expressed as mean±S.E., n=6. [b]$P<0.01$ vs control group; [c]$P<0.05$ vs Hcy group (Newman–Keuls test).

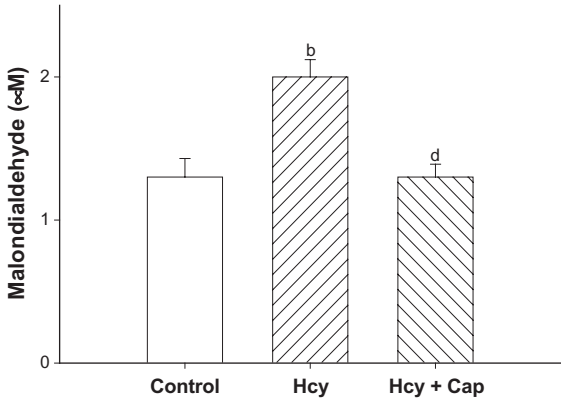

Fig. 4. Effect of captopril treatment on serum malondialdehyde concentrations in rats with homocysteine intravenous injection. Concentrations of malondialdehyde (MDA) in serum from control, homocysteine (Hcy), and homocysteine plus captopril (Hcy+Cap) group rats were detected by thiobarbituric acid method described by Yagi. Data are expressed as mean±S.E., n=6. ^{b}P<0.01 vs control group; ^{d}P<0.01 vs Hcy group (Newman–Keuls test).

of superoxide dismutase, an antioxidant enzyme, in liver was significantly decreased in homocysteine-treated group compared with control group. After treatment with captopril for 4 weeks, both the elevation of MDA concentration in serum and the reduction of superoxide dismutase activity in liver induced by homocysteine were reversed (Figs. 4 and 5).

3.4. Serum angiotensin converting enzyme activity and creatinine levels

The serum angiotensin converting enzyme activity and creatinine levels were not significantly different between control, homocysteine, and homocysteine plus captopril group (Table 2).

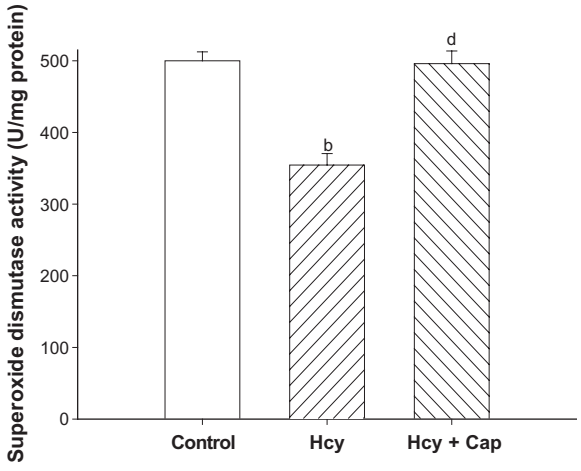

Fig. 5. Effect of captopril treatment on superoxide dismutase activity in liver of rats with homocysteine intravenous injection. Superoxide dismutase activity in liver of control, homocysteine (Hcy), and homocysteine plus captopril (Hcy+Cap) group rats was assayed by monitoring the inhibition of the autoxidation of hydroxylamine. Data are expressed as mean±S.E., n=6. ^{b}P<0.01 vs control group; ^{d}P<0.01 vs Hcy group (Newman–Keuls test).

Table 2
Serum angiotensin converting enzyme activity and creatinine levels in control, homocysteine (Hcy), and captopril-treated (Hcy+Cap) rats

	Control	Hcy	Hcy+Cap
Angiotensin converting enzyme activity (nM/min)	51.8±3.3	49.9±5.2	55.8±4.3
Creatinine (μM)	57.8±1.1	60.6±2.5	56.4±5.7

Data are expressed as mean±S.E., n=6. All P>0.05 (Newman–Keuls test).

4. Discussion

The major findings of the present study are as follows: (1) intravenous injection of homocysteine to normal rats markedly inhibited endothelium-dependent relaxation response to acetylcholine, which was in parallel with the elevation of serum ADMA levels as well as the reduction of serum nitrite/nitrate contents, (2) and these effects were also accompanied by the increase of serum MDA concentrations as well as the decrease of superoxide dismutase activity in the liver; (3) chronic treatment with captopril not only significantly attenuated the inhibition of endothelium-dependent relaxation but also prevented the elevation of serum ADMA concentrations, and simultaneously reversed the changes of serum MDA, nitrite/nitrate levels, and liver superoxide dismutase activity induced by homocysteine. These results suggest that captopril-induced improvement of impaired endothelium-dependent relaxation by homocysteine is related to reducing endogenous ADMA, which may be secondary to its antioxidant property.

Endothelial dysfunction is considered as an early step in the pathogenesis of atherosclerosis. Homocysteine-induced endothelium-dependent vasodilation dysfunction may predispose a person to vascular diseases. Endothelium-derived NO has been recognized as a potent, endogenous anti-atherogenic molecule. In addition to mediating the endothelium-dependent vasorelaxation, NO interferes with key events in the development of atherosclerosis, such as oxidative damage to endothelium, monocyte adhesion and migration to vessel wall, platelet aggregation, and vascular smooth muscle proliferation (Cooke, 2000). Our previous study showed that exposure of rat aortic rings to homocysteine in vitro caused a significant attenuation of endothelium-dependent relaxation response to acetylcholine (Fu et al., 2003). In the present study, we demonstrated that intravenous injection of homocysteine to rats also markedly decreased their endothelium-dependent relaxation of aortas. Similar results have been observed in rats with continuous venous infusion of homocysteine (Fu et al., 2002). Furthermore, reduced endothelium-dependent vasodilatation in both large conduit arteries and resistance vessels was observed in monkeys and humans with hyperhomocysteinemia (Böger et al., 2000, 2001; Stühlinger et al., 2003;

Sydow et al., 2003). Therefore, homocysteine is an important factor for endothelial dysfunction associated with atherosclerosis.

The mechanism by which homocysteine induces endothelial dysfunction is not elucidated. It is well known that endothelium-derived NO is synthesized from L-arginine by NOS, and ADMA, an L-arginine analogue, has been recognized as an endogenous competitive inhibitor of NOS, which reduces the synthesis of NO and subsequently results in endothelial dysfunction. Our previous studies showed that endothelial dysfunction in hyperlipidemic rabbits and diabetic and aging rats are associated with the elevation of serum ADMA contents (Yu et al., 1994; Xiong et al., 1996, 1997, 2001, 2003). Consistent with the previous report (Duan et al., 2000), the present study revealed that in addition to inhibiting endothelium-dependent relaxation of aorta response to acetylcholine, vein injection of homocysteine to rats also significantly increased serum ADMA levels and decreased serum nitrite/nitrate contents, the stable end products of NO, and reflecting NO synthesis. Recently, several scholars reported similar results in the studies performed in monkeys and humans with hyperhomocysteinemia (Böger et al., 2000, 2001; Stühlinger et al., 2003; Sydow et al., 2003). Furthermore, incubation of cultured endothelial cells with homocysteine also induced an accumulation of endogenous ADMA in the media (Böger et al., 2000, 2001; Stühlinger et al., 2001). Taken together, these studies suggest that elevated endogenous ADMA probably contributes to the endothelial dysfunction induced by hyperhomocysteine.

Malondialdehyde has extensively been used as a marker of lipid peroxidation, and superoxide dismutase is a ubiquitous enzyme that scavenges superoxide anions. Although measurement of MDA is not completely specific, it is the most commonly used parameter of lipid peroxidation. Furthermore, we assayed the superoxide dismutase activity in liver that is an antioxidant enzyme in order to complement MDA measurements. Our previous studies have demonstrated that increases in serum ADMA levels are accompanied by the elevation of MDA concentrations in hyperlipidemic rabbits and diabetic and aging rats (Yu et al., 1994; Xiong et al., 1996, 1997, 2001, 2003). Supplement with the antioxidant vitamin E to hyperlipidemic rabbits can decrease MDA levels concomitantly with reduction in serum concentrations of ADMA (Xiong et al., 1996). Ito et al. have also reported that incubation of human umbilical vein endothelial cells with oxidized low-density lipoprotein (ox-LDL) increases the ADMA content in culture medium (Ito et al., 1999). Administration of exogenous LDL to normal animals caused a significant elevation of serum ADMA levels (Jiang et al., 2002). The accumulation of endogenous ADMA either in culture medium of endothelial cells or in serum of experimental animals induced by both homocysteine and ox-LDL could be prevented with the use of antioxidants (Jiang et al., 2002; Stühlinger et al., 2001). These studies strongly support the proposal that the elevation of endogenous ADMA concentration is closely related to the increase in lipid peroxide levels. It is well documented that hyperhomocysteinemia is associated with enhanced oxidative stress, as evidenced by increased oxygen free radicals and/or decreased antioxidant defense mechanisms (Huang et al., 2001; Sydow et al., 2003). The present study demonstrated that injection of homocysteine to rats not only elevated the serum ADMA levels but also increased the serum MDA content and decreased superoxide dismutase activity in liver. These results indicate that the elevation of endogenous ADMA levels in homocysteine-treated rats may be secondary to the increase of lipid peroxide.

Captopril is an SH-containing angiotensin converting enzyme inhibitor and is identified to possess potent antioxidant activity, which can scavenge oxygen free radicals or oxidant and inhibit lipid peroxidation. A number of studies have shown that captopril presents the protective effects against endothelium damage induced by hypercholesterolemia, oxygen free radicals, and cigarette smoke extract (Hernandez et al., 1998; Liao and Chen, 1992; Ota et al., 1997), and the beneficial effect of captopril on endothelium is ascribed to its antioxidant activity. Recently, we found that captopril can also prevent homocysteine-induced impairment of endothelium-dependent relaxation in isolated rat aortic rings in vitro (Fu et al., 2003). In the present study, we demonstrated that chronic captopril treatment to rats with daily intravenous injection of homocysteine not only improved the impaired endothelium-dependent relaxation, but also prevented the elevation of serum ADMA levels as well as reduction of serum NO contents. In addition, captopril also concomitantly decreased the serum levels of MDA derived from lipid peroxidation, and increased liver superoxide dismutase activity in rats with homocysteine administration. These results provide the first evidence that the beneficial effect of captopril on homocysteine-induced endothelial dysfunction is related to the reduction of endogenous ADMA. Moreover, our results also indicate that captopril reducing endogenous ADMA may be secondary to its antioxidant activity. Napoli et al. have recently reported that another new sulfhydryl angiotensin converting enzyme inhibitor zofenopril reduces oxidative stress, decreases ADMA concentrations, and improves the NO pathway in patients with essential hypertension (Napoli et al., 2004). This result also indirectly supports our results.

Captopril is a short-acting angiotensin converting enzyme inhibitor. In order to determine whether the protective effect of captopril on endothelial function injury induced by homocysteine is due to its property of inhibiting systemic angiotensin converting enzyme activity, we measured the serum angiotensin converting enzyme activities in rats of control, homocysteine, and homocysteine plus captopril group in the present study. The results demonstrated that there were no significant differences in

serum angiotensin converting enzyme activity among the 3 groups. These results indicate that the beneficial effects of captopril on endothelial dysfunction induced by homocysteine are independent of its inhibiting action on systemic angiotensin converting enzyme. The dosage of captopril (3 mg/kg/day) used in our present study is in the range of the dosages in many of other researches (Nelissen-Vrancken et al., 1992; Plante et al., 1988). As we all know, captopril will dissociate from angiotensin converting enzyme during long-time storage serum, leading to underestimation of the degree of angiotensin converting enzyme inhibition. In our study, however, we took precautions to minimize this effect. All samples were immediately stores at −70 °C and were assessed in <48 h. So under dosage and timing of blood samples cannot explain the lack of inhibition of serum angiotensin converting enzyme activity in captopril-treated rats. The other authors have also reported that chronic captopril treatment had no significant inhibition of serum angiotensin converting enzyme activity in hypertensive patients and experimental heart failure animals (Rodriguez et al., 1986; Hirsch et al., 1992). Because blocking angiotensin II production by angiotensin converting enzyme inhibition may induce angiotensin converting enzyme mRNA expression (Schunkert et al., 1993), the latter can compensate for the inhibition of angiotensin converting enzyme activity as a consequence (Hirsch et al., 1992). However, Rolland et al. showed that combinative treatment with captopril and hydrochlorothiazide inhibited serum angiotensin converting enzyme activity in hyperhomocysteinemia minpigs although they did not find a significant difference in serum and lung angiotensin converting enzyme activity between hyperhomocysteinemic minpigs and control minpigs (Rolland et al., 1995). These conflicting results may be due to different animals or different drugs combination. It is not certain whether the protective effect of captopril is sulfhydryl-dependent, because we did not compare the effect of the angiotensin converting enzyme inhibitor without a sulfhydryl moiety in this study. However, Delles and Ito have recently reported that enalapril and perindopril, both non-SH-containing angiotensin converting enzyme inhibitors, also significantly attenuated the elevation of serum ADMA levels and improved endothelial function in patients with hypertension or diabetes mellitus (Delles et al., 2002; Ito et al., 2002). These results suggest that angiotensin converting enzyme inhibitors possess the properties of decreasing ADMA concentrations and improving the NO pathway, which may be sulfhydryl-independent. It is known that chronic administration of angiotensin converting enzyme inhibitors can have effects on vascular angiotensin converting enzyme at doses that are not effective to abolish systemic angiotensin converting enzyme activity. Because the vascular specimens were used for the assay of endothelium-dependent and -independent relaxation function, we study angiotensin converting enzyme activity in serum instead of vascular tissue. So it is possible that vascular angiotensin converting enzyme activity in this study is suppressed by captopril. Furthermore, eprosartan, an angiotensin II type I receptor blocker, has recently been shown to reduce ADMA levels in hypertensive patients, suggesting that a reduction in angiotensin II receptor stimulation plays a role in reducing ADMA levels (Delles et al., 2002). Angiotensin II stimulates the vascular production of superoxide anion by NADPH oxidase (Zhang et al., 1999), which might contribute to increasing ADMA levels (Delles et al., 2002; Ito et al., 2002). We speculate that captopril treatment may inhibit vascular topical angiotensin converting enzyme pathway and subsequently decrease oxidative stress to contribute to preventing the elevation of endogenous ADMA concentrations. However, more studies are needed to assess the direct effect of angiotensin II type I receptor blocker on serum ADMA levels in hyperhomocysteinemia.

Asymmetric dimethylarginine is excreted via the kidney and accumulated in patients with chronic renal failure (Vallance et al., 1992b). To determine whether the differences in serum ADMA levels observed in the 3 groups of rats were due to changes in renal function, we assayed serum creatinine concentrations as an indicator of renal function. As results show, serum creatinine levels were not remarkably different among the 3 groups. Similar results have been seen in hyperhomocysteinemia minpigs (Rolland et al., 1995). Therefore, these results indicate that the differences in serum ADMA concentrations in the present study are not due to renal function changes.

In conclusion, the present study suggests that captopril protects aortic endothelium against injury due to homocysteine in rats, and that the beneficial effect of captopril is related to reduction of ADMA concentration, which may be secondary to the antioxidative action of captopril. Therefore, captopril is an effective pharmacological approach to preventing the elevation of endogenous ADMA levels.

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