Renin-Angiotensin System is Involved in the Mechanism of Increased Serum Asymmetric Dimethylarginine in Essential Hypertension

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Endothelium-dependent/nitric oxide (NO)-mediated vasodilation is impaired in hypertensive individuals. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, is synthesized by many types of cells including vascular endothelial cells. The serum level of ADMA is elevated in patients with essential hypertension, but the mechanism for this increase is unknown. Therefore, the present study examined whether the renin–angiotensin system (RAS) is involved. Patients with essential hypertension [systolic blood pressure (BP) >160 mmHg and/or diastolic BP >95 mmHg] were randomized to an angiotensin-converting enzyme (ACE) inhibitor treatment group (perindopril, 4 mg/day for 4 weeks, n=7), an angiotensin II type 1 (AT1) receptor antagonist treatment group (losartan, 50 mg/day for 4 weeks, n=7) or a β -blocker treatment group (bisoprolol, 5 mg/day for 4 weeks, n=7). Before and after the treatment, BP, serum concentration of ADMA and plasma concentration of von Willebrand factor (vWF, a biological marker of endothelial injury) were measured. Perindopril, losartan and bisoprolol decreased BP to a similar extent, and either perindopril or losartan, but not bisoprolol, significantly decreased serum ADMA and plasma vWF. These findings suggest that the RAS may contribute to the mechanism of increased serum ADMA as well as to the endothelial injury observed in hypertensive patients. The vasculoprotective actions of ACE inhibitors or AT1 receptor antagonists may be explained at least in part by amelioration of the endothelial injury through a decrease in the serum ADMA concentration. (*Jpn Circ J* 2001; 65: 775–778)

Key Words: Angiotensin II; Angiotensin-converting enzyme; Endothelial injury; Nitric oxide synthase; Von Willebrand factor

Endothelium-derived nitric oxide (NO) is a potent vasodilator that plays a critical role in regulating vascular resistance and flow! In addition, NO inhibits key processes in atherogenesis, such as monocyte adhesion, platelet aggregation and vascular smooth muscle proliferation²; and in disorders associated with atherosclerosis (eg, hypertension, hypercholesterolemia, diabetes mellitus), there is less endothelium-dependent NO-mediated vasodilation². Impairment of the NO synthase system adversely affects vascular reactions and blood flow and, in addition, because NO inhibits key processes in atherogenesis, an NO deficiency state may contribute to the initiation and the progression of atherosclerosis^{2,3}. Although the mechanisms of endothelial vasodilator dysfunction are likely to be multifactorial, one contributing abnormality appears to be increased levels of asymmetric dimethylarginine (ADMA)^{4,5}.

ADMA is an endogenous competitive inhibitor of NO synthase⁶ and serum or plasma levels of ADMA are elevated in individuals with hypertension⁷, hypercholesterolemia⁴, diabetes mellitus⁵, peripheral arterial occlusive

disease⁸ or congestive heart failure⁹. Elevation of ADMA is associated with impaired endothelium-dependent NO-mediated vasodilation in the brachial artery⁴ and is also significantly correlated with the intima–media thickness of the carotid artery, a non invasive measure of atherosclerosis⁵. ADMA is thought to derive from proteins that have been posttranslationally methylated and subsequently hydrolyzed to release ADMA¹⁰; a number of cells elaborate ADMA, including vascular endothelial cells¹¹. ADMA may be excreted in the urine or metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH)¹² to L-citrulline and dimethylamine. We have recently reported that lipid¹³ or hyperglycemia-induced¹⁴ dysregulation of DDAH may play an important role in the elevation of ADMA in hypercholesterolemia or diabetes mellitus, respectively. DDAH has sulfhydryl groups in its structure¹² and it was recently reported that the antioxidant pyrrolidine dithiocarbamate (PDTC) inhibited homocysteine-induced ADMA accumulation in conditioned medium of human endothelial cells by reversing the decreased activity of DDAH¹⁵. However, it is not understood how the serum concentration of ADMA becomes elevated in humans in vivo.

It has been reported that angiotensin-converting enzyme (ACE) inhibitors improve endothelial function in hypertensive patients¹⁶ suggesting that the activation of the renin–angiotensin system (RAS) may contribute to the endothelial dysfunction in those individuals. Furthermore, angiotensin II (AII) type I (AT1) receptor antagonists may also have this vasculoprotective action¹⁷ and they are now widely

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used for cardiovascular disorders. However, it is not understood how these drugs modify the RAS to improve vascular function, although recently, they have been shown to reduce free radical concentrations in patients with coronary artery disease.¹⁸

Therefore, we investigated whether the RAS and/or oxidative stress is involved in the mechanisms of ADMA elevation and endothelial injury in hypertensive patients.

Methods

Patients

Untreated patients with essential hypertension (systolic blood pressure (BP) >160 mmHg and/or diastolic BP >95 mmHg), who gave informed consent to be enrolled in the study, which has been approved by the hospital's Ethics Committee, were randomly assigned to the following treatment groups: (1) an ACE inhibitor group (perindopril, 4 mg/day, n=7), (2) an AT1 receptor antagonist group (losartan, 50 mg/day, n=7) or (3) a β -blocker (bisoprolol, 5 mg/day, n=7). All patients had normal renal function. Patients with hypercholesterolemia (serum cholesterol >240 mg/dl), diabetes mellitus (fasting serum glucose >120 mg/dl), suspicion of coronary artery disease or peripheral arterial occlusive disease, a history of congestive heart failure or stroke were excluded, because each one of those conditions may increase serum ADMA levels.

Evaluation

Before and 4 weeks after the treatment, the following measurements were performed.

Fig 1. Changes of mean blood pressure (BP) (Top), serum asymmetric dimethylarginine (ADMA) concentration (Middle) and plasma von Willebrand factor (vWF) level (Bottom). P, L and B, treatment with perindopril, losartan and bisoprolol, respectively. In each treatment group, open and closed bars indicate before and 4 weeks after the treatment, respectively.

(1) BP was measured in the seated positions after several deep breaths.

(2) Serum ADMA concentration was measured by high-performance liquid chromatography. The variability of the method was less than 7%, and the detection limit of the assay was 0.15 μ mol/L.

(3) Plasma concentration of von Willebrand factor (vWF, a marker of endothelial injury) was measured by an enzyme immunoassay technique. It has been reported that an increased level of vWF is associated with impaired endothelium-dependent vasodilation¹⁹ and that it can predict the appearance or progression of atherosclerosis in patients with hypertension.²⁰

(4) Serum ACE activity, plasma AII concentration and serum malondialdehyde-modified low density lipoprotein (MDA-LDL) (a marker of oxidative stress) were also determined.

The within-run coefficient of variation for vWF or MDA-LDL was 2–3% or 4–8%, respectively, and the detection limit of the assay for MDA-LDL was 12.5 U/L.

Statistical Analysis

Values are expressed as mean $\pm$ SE. Differences among treatment groups were tested by ANOVA. Between before and after the treatment, differences were evaluated by paired Students' t test; $p < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the patients are shown in Table 1. There were no significant differences between the 3 treatment groups in age, sex, BP, heart rate, serum cholesterol, high density lipoprotein cholesterol or fasting glucose levels at baseline. Before the treatment, there were no significant differences in serum ADMA, plasma vWF, serum ACE activity, plasma AII or serum MDA-LDL.

Four weeks after the treatment, mean BP was significantly decreased to the same extent by all 3 treatments (Fig 1), and similarly for systolic and diastolic BP (data not shown). Only perindopril or losartan, but not bisoprolol, significantly decreased both serum ADMA concentrations and plasma vWF levels (Fig 1). Serum ACE activity was significantly suppressed only by perindopril (data not shown), and plasma AII concentration was significantly increased only by losartan (data not shown). Serum MDA-LDL was significantly decreased only by losartan (from 158 ± 32 to 130 ± 27 U/ml, $p < 0.05$).

Discussion

The salient findings of this study are: (1) serum ADMA concentration was significantly decreased by an ACE inhibitor (perindopril) or an AT1 receptor antagonist (losartan), but not by a β -blocker (bisoprolol), and (2) plasma vWF, a marker of endothelial injury, was also significantly decreased by either perindopril or losartan, but not by bisoprolol, although these 3 agents decreased BP to the same extent.

This study provides insight into a novel mechanism by which activation of the RAS may disturb both the NO synthase pathway and endothelial function in essential hypertension.

Increased levels of ADMA, the endogenous NO synthase inhibitor, are observed in individuals with hypertension.⁷

Table 1 Baseline Characteristics of Patients

	Perindopril	Losartan	Bisoprolol
Age (years)	58±3	59±4	53±2
M/F	3/4	4/3	3/4
Systolic BP (mmHg)	165±2	174±4	171±5
Diastolic BP (mmHg)	97±2	103±3	104±2
Mean BP (mmHg)	118±2	127±3	126±2
Heart rate (beats/min)	65±3	69±4	76±7
Total cholesterol (mg/dl)	211±16	201±15	225±22
HDL cholesterol (mg/dl)	58±6	60±4	58±6
Triglyceride (mg/dl)	73±11	92±13	119±17
Glucose (mg/dl)	100±3	99±5	110±3
Serum ADMA (μmol/L)	0.43±0.02	0.51±0.02	0.42±0.04
Plasma vWF (%)	170±20	125±16	143±16
Serum ACE activity (IU/L at 37°C)	10.9±1.2	12.7±1.8	9.3±2.3
Plasma AII (ng/ml)	23±13	12±2	17±3
Serum MDA-LDL (U/L)	115±32	158±32	118±22

BP, blood pressure; ADMA, asymmetric dimethylarginine; vWF, von Willebrand factor; ACE, angiotensin-converting enzyme; AII, angiotensin II; MDA, malondialdehyde-modified.

and may account in part for the endothelial vasodilator dysfunction observed in this condition. Increased ADMA levels are associated with reduced NO elaboration in both hypercholesterolemic⁴ and atherosclerotic patients⁸ as judged by reduced nitrate excretion and impaired endothelium-dependent, NO-mediated forearm vasodilation.

It has been reported that in healthy humans the serum ADMA level is around 0.5 μmol/L.⁵ In the present study, the serum ADMA level was not elevated, which may be because the severity or the duration of hypertension also contributes to the elevation of serum ADMA. Further studies are needed to examine the effects of ACE inhibitors or AT1 receptor antagonists on ADMA levels in patients with severe hypertension or with other risk factors for atherosclerosis.

We measured plasma vWF as a marker of endothelial injury, but vWF is thought to be increased in disorders with endothelial dysfunction.²¹ Therefore, one of the limitations of this study is that endothelial function itself was not evaluated, for example, by measurement of flow-dependent vasodilation of brachial artery using an ultrasound technique.²²

Although the difference between the 3 treatment groups in serum ADMA concentration or plasma vWF level before the treatments was not statistically significant (Table 1), there was some degree of difference among them. Furthermore, there was no correlation between ADMA and vWF, and the decrease of ADMA by perindopril or losartan was only about 10%. These results suggest that some factors other than those measured in this study, such as serum homocystein levels etc, might be related to endothelial function, and that the elevation of ADMA is just one of the key factors contributing to endothelial dysfunction.

We recently demonstrated that lipid-¹³ or hyperglycemia-induced¹⁴ dysregulation of DDAH, a degradation enzyme of ADMA, plays an important role in the elevation of ADMA in hypercholesterolemia or diabetes mellitus, respectively. Oxidative stress has been suggested as one of the mechanisms of its decreased activity.¹⁵ In the present study, we measured serum MDA-LDL as a marker of oxidative stress. Losartan, but not perindopril or bisoprolol, significantly decreased serum MDA-LDL, suggesting that losartan might decrease serum ADMA by reducing oxidative stress, and that other mechanisms are involved in the perindopril-induced decrease of serum ADMA. Further

studies are needed to clarify the role of DDAH in the effects of ACE inhibitors or AT1 receptor antagonists on serum ADMA levels in hypertensive patients.

ADMA is widely distributed in tissues¹² and may be the mechanism for controlling NO synthesis in physiological and/or pathological states. We have demonstrated that the RAS may be involved in the elevation of serum ADMA in essential hypertension and our results suggest that the vasculoprotective actions of ACE inhibitors or AT1 receptor antagonists can be explained at least in part by the amelioration of endothelial injury (dysfunction) through the decreased serum ADMA concentration.

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