Original Article

Effects of Angiotensin Converting Enzyme Inhibitor and Angiotensin II Receptor Antagonist Combination on Nitric Oxide Bioavailability and Atherosclerotic Change in Watanabe Heritable Hyperlipidemic Rabbits

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We investigated the effects of co-administration of an angiotensin-converting enzyme inhibitor (ACEI) and angiotensin type 1 receptor blocker (ARB) on nitric oxide (NO) bioavailability in genetically hyperlipidemic rabbits with our newly developed NO sensor. Plasma NO was measured using the new NO sensor in the abdominal aorta of anesthetized Watanabe heritable hyperlipidemic (WHHL) rabbits. Acetylcholine (ACh)-stimulated (20 μg in 5 min into the aortic arch) NO production was recorded after an 8 week per os pretreatment with 1) vehicle (control), 2) the ACEI enalapril (E: 3 mg/kg/day), 3) the ARB losartan (L: 30 mg/kg/day) and 4) enalapril (1.5 mg/kg/day) + losartan (15 mg/kg/day) (E+L). Intra-aortic infusion of ACh produced an increase in plasma NO concentration, which was significantly greater with all the drug treatments than with the control. E increased ACh-induced NO significantly more than L (by 6.9 nmol/L, and 4.7 nmol/L, respectively). E+L increased ACh-induced NO by 9.5 nmol/L, significantly more than either E or L. Plasma peroxynitrite concentration was 1.2 pmol/mg protein in the control group and significantly less than in the E- and L-group. The lowest peroxynitrite concentration was observed in the E+L group (0.5 pmol/mg protein), which was significantly lower than in the E-group and the L-group. Optical coherence tomography and histology of the thoracic aorta revealed that the plaque area decreased significantly more with the combination than with the monotherapy ($p<0.01$). In conclusion, the combined treatment with an ACEI and an ARB may have additive protective effects on endothelial function as well as atherosclerotic change. (*Hypertens Res* 2008; 31: 575–584)

***Key Words*: nitric oxide, endothelial function, renin angiotensin system**

Introduction

Endothelium dysfunction has been characterized by decreased nitric oxide (NO) synthesis or reduced NO bioavailability, which relates to inflammation, proliferation of smooth muscle cells, deposition of extracellular matrix, vasoconstriction, and a prothrombotic state within the vessel lumen (*1*, *2*). Although NO has previously been considered to be immediately inactivated in the bloodstream by dissolved

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Received June 7, 2007; Accepted in revised form October 4, 2007.

Table 1. Final Hemodynamic Data by Group

	MAP, mmHg	HR, bpm	BW, kg
Control	73.5±1.1	169±2	2.34±0.06
Enalapril	66.5±1.2	171±1	2.31±0.03
Losartan	67.1±1.5	167±2	2.30±0.04
Enalapril+losartan	66.8±1.6	168±4	2.30±0.03

Data are the mean±SEM. MAP, mean aortic pressure; HR, heart rate; BW, body weight.

Table 2. Lipidemic Effects of Treatment in WHHL Rabbit

	TC (mg/dL)		TG (mg/dL)	
	Basal	8 week	Basal	8 week
Control	678±24	592±16	246±18	216±15
Enalapril	688±19	600±22	239±16	210±13
Losartan	675±21	585±18	225±17	205±14
Enalaprl+losartan	670±22	566±16	238±14	207±12

Data are the mean±SEM. WHHL, Watanabe heritable hyperlipidemic; TC, total cholesterol; TG, triglyceride.

oxygen, oxyhemoglobin or oxygen radical species (*3*, *4*), mounting experimental and clinical evidence has suggested that NO remains in an active form causing vasodilatory responses (*5*, *6*). Thus, reduced NO bioavailability and impaired endothelium-dependent vasomotion have been linked to cardiovascular events. Recently, we have developed a new catheter-type NO sensor, which can directly measure intra-arterial NO concentration *in vivo* (*7*). Using the NO sensor, which can detect changes in plasma NO, we have shown that in anesthetized dogs the intracoronary injection of acetylcholine (ACh) increases coronary flow velocity and with some delay plasma NO in the coronary sinus (*8*). Measuring NO with this technique in the abdominal aorta of normal rabbits, we have shown more recently that the chronic application of angiotensin II (Ang II) reduces the plasma NO concentration and increases the plasma peroxynitrite concentration. The Ang II–mediated decrease in basal and ACh-induced NO production and the increase in nitrosative stress were significantly suppressed by the pre-treatment with the Ang II–receptor antagonist valsartan (*9*).

In several clinical studies, both angiotensin-converting enzyme (ACE) inhibitors (ACEIs) and angiotensin type 1 (AT_1) receptor blockers (ARBs) have been shown to improve endothelial function in patients with cardiovascular risk factors or coronary disease (*10*–*12*). However, the effects of these agents on the bioavailability of NO and vascular remodeling has not been investigated in details. In the present study, the effects of the ACEI enalapril, the ARB losartan, and the two drugs in combination on NO bioavailability measured with our sensor, atherosclerotic change and plasma peroxynitrite level were investigated in experimental hyperlipidemic animal model.

Methods

Catheter-Type NO Sensor

The integrated architecture and performance of the catheter-type NO sensor have been described previously (*7*–*9*). In brief, by using an NO sensor (amino-700 XL; Innovative Instruments, Tampa, USA; 700 μm in diameter at the detection tip), the oxidative current of NO was measured with an NO monitor (model inNO-T; Innovative Instruments). For each sensor, the baseline (zero level) was set arbitrarily using the amperometric method and calibrated with NO-saturated pure water. A change in the current form from the baseline is used and is expressed as "change in NO concentration (nmol/L)" as previously described (*7*–*9*).

Animal Preparation

We confirmed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. In addition, the study protocol was approved by the Institutional Animal Care and Use Committee of the Wakayama Medical University. Male Watanabe heritable hyperlipidemic (WHHL) rabbits (3 months old) were obtained from the Centre de Production Animals (CPA). The rabbits were maintained on tap water and a standard diet, and randomized into one of four groups that received oral administration of 0.5% carboxymethylcellulose sodium as a vehicle (vehicle group), 3 mg/kg/day of enalapril (enalapril group), 30 mg/kg/day of losartan (losartan group), or 1.5 mg/kg of enalapril plus 15 mg/kg of losartan (enalapril-losartan group) daily for 8 weeks. In previous studies, early atherosclerotic lesions have been noted in the aortae of WHHL rabbits as early as 3 months, and lesion formation was progressively accelerated with aging at 6 months (*13*, *14*). In the present study, we hypothesized that both ACEIs and ARBs have preventive effects on dysfunction of NO bioavailability and atherosclerosis in WHHL rabbits. To test this hypothesis, we administered enalapril or losartan to 3-month-old WHHL rabbits for a period of 8 weeks, which corresponded to the period from early to advanced atherosclerotic lesions in WHHL rabbits. Rabbits were anesthetized with xylazine (10 mg/kg intramuscularly), ketamine (50 mg/kg intramuscularly), and pentobarbital sodium (10 mg/kg intravenous), followed by administration of heparin (1,000 units intravenous) as an anticoagulant. A catheter for ACh and N^G-methyl-L-arginine (L-NMMA) infusion was placed in the aortic arch from the external carotid artery. The NO sensor was inserted through the left femoral artery and placed in the abdominal aorta. Aortic blood pressure was simultaneously monitored through a stiff cannula with a strain gauge pressure transducer (Nihon Kohden, Tokyo, Japan). Blood samples were collected with EDTA (1 mg/mL) from the ear artery. Plasma was separated

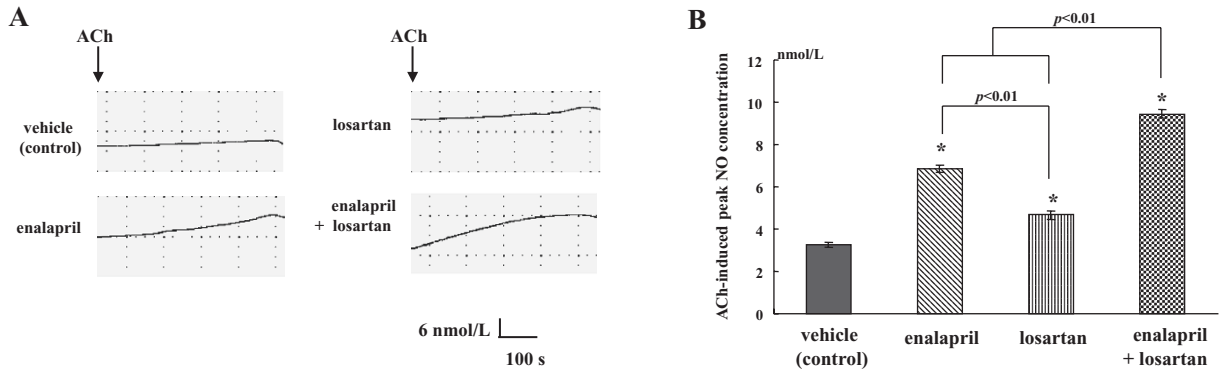

Fig. 1. *ACh-induced plasma NO production in the aortae of WHHL rabbits. A: Typical change in plasma NO concentration induced by 20 μg/kg of ACh infusion (5 min) in the thoracic aortae of WHHL rabbits (n = 6 per group) treated with vehicle (control), enalarapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day) for 8 weeks. B: Mean NO concentration change (nmol/L) at the peak response to acetylcholine (ACh) among the above four groups of rabbits (n = 6 each). Data are shown as the means ± SEM.*

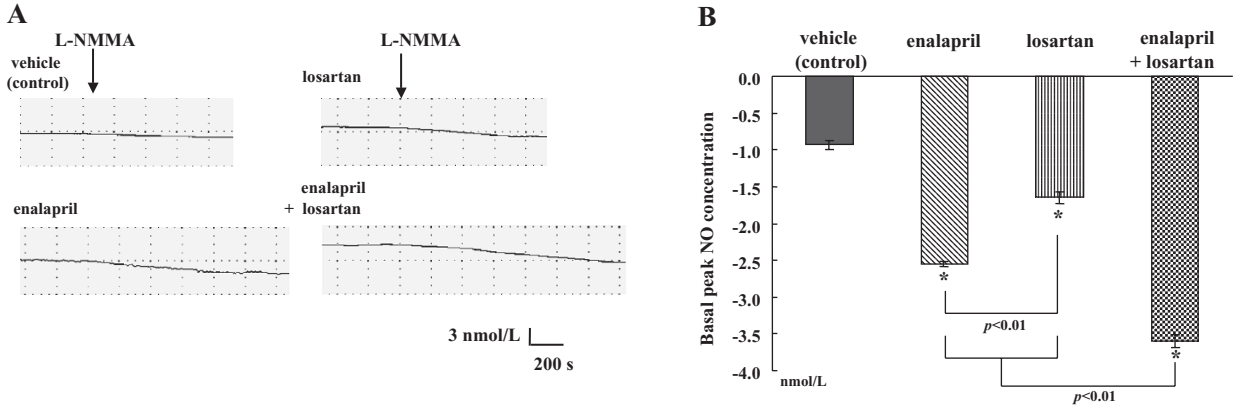

Fig. 2. *Basal plasma NO concentration. A: Typical changes in plasma NO concentration of the thoracic aortae under L-NMMA infusion (10 min) in WHHL rabbits treated with vehicle (control), enalapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day) for 8 weeks. It should be noticed that under a resting condition, the NO sensor detected only a fluctuation. B: Mean basal plasma NO concentration of the thoracic aorta at the peak response to 5 mg/kg of L-NMMA infusion (10 min) in the above-described groups of rabbits (n = 6 each). Data are shown as the means ± SEM (n = 6).*

and measured for total cholesterol (TC) and triglyceride (TG) levels using a commercially available kit (Wako Pure Chemical Industries, Osaka, Japan). In addition, to investigate the short-term effects of the ACEI enalapril, the ARB losartan, or the two in combination on the NO bioavailability, the animals received vehicle, enalapril, or enalapril plus losartan for 24 h as described above.

Measurement of NO Production

To measure the endothelium-dependent NO production, ACh (20 μg/kg) was administered for 5 min. To measure the basal NO production, an NO synthase inhibitor, 5 mg/kg of L-NMMA was infused at 1 mL/min for 10 min (*15*, *16*). Plasma NO concentration in the abdominal aorta was monitored over the entire time course.

Measurement of Plasma Nitrotyrosine

Peroxynitrite is a strong oxidant formed in the reaction between NO and superoxide under atherosclerotic stimuli. The peroxynitrite subsequently reacts with proteins, resulting in nitrotyrosine. As a stable end product of peroxynitrite-mediated oxidation/nitration, nitrotyrosine can be used as a surrogate index of elevated superoxide production. The binding of plasma nitrotyrosine to proteins was measured using an

NWLSS™ nitrotyrosine enzyme–linked immunoassay (ELISA) kit (Northwest Life Science Specialties, LLC, Vancouver, USA) according to the manufacturer's protocol (*17*).

Optical Coherence Tomography Imaging

The plaque area of the thoracic aorta was evaluated with optical coherence tomography (OCT) imaging. After the animals were sacrificed, the aorta from the aortic arch to the lower thoracic aorta was removed. The surrounding soft tissues were dissected from each specimen. The branches were tied off with sutures, and the distal end of the artery was plugged with a large cork. A 7F sheath was sewn into the proximal end of the artery to complete the closed system. Saline (0.9%) kept at a temperature of 37°C was infused through the side arm of the sheath. The pressure inside the artery was maintained at a physiologic level (60–80 mmHg) with a syringe-manometer connected to the infusion pump. The intravascular OCT catheter (ImageWire; LightLab Imaging, Westford, USA) was inserted through the diaphragm of the sheath. Serial images of the OCT were obtained using an automatic pullback device at a rate of 0.5 mm/s. The obtained OCT images were processed and analyzed using appropriate software from LightLab Imaging for off-line analysis. According to the method used in the previous reports (*16*), lipid was semiquantified as the number of involved quadrants on the cross-sectional OCT image. The lipid-rich plaque was defined as the lipid content in more than 2 quadrants in any of the image. OCT-identified plaque lesions were also quantified by the degree of arc in the involved segments in the cross-sectional OCT image along the thoracic aorta (*18*). We also examined for the presence of thrombus. A thrombus was defined as an irregular mass with a diameter ≥250 μm protruding into the lumen. Overall, 200 OCT images for each specimen were analyzed by two independent observers (*18*).

Histological Analysis

After OCT imaging, each artery was pressure-fixed in 10% neutral buffered formalin for 48 h. After the fixation and standard paraffin embedding, serial cross sections were processed for general histological staining with hematoxylin-eosin (H&E). The method used for determining the ratio of the intimal area to the medial area of the aorta, which is used as a measure of atherosclerotic burden, has been previously described (*19*). Six sections from each rabbit aorta were used to determine the intimal area–medial area ratio. We also examined the distribution of macrophages as described by Imanishi *et al*. (*19*). Aortic cross sections (5 μm) were stained immunohistochemically with monoclonal antibody recognizing macrophage (RAM-11) antibody (DAKO, Carpinteria, USA) and developed with a Vectorstain Universal ABC kit (Vector Laboratories, Burlingame, USA, Cat. No. PK-6200).

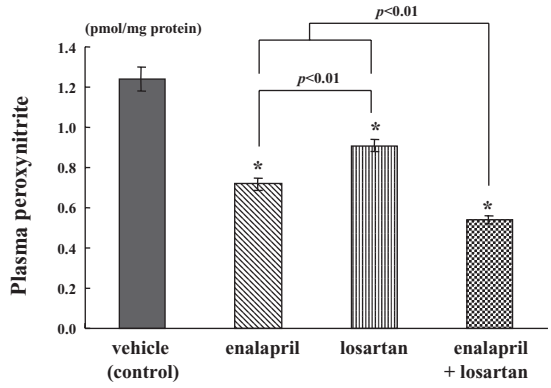

Fig. 3. *Mean plasma peroxynitrite concentration in the aortae of rabbits treated with vehicle (control), enalapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day) for 8 weeks. Data are expressed as the means±SEM (n=6).*

Statistical Analysis

All data were expressed as the mean±SEM based on six independent experiments. Differences between groups were analyzed by ANOVA followed by Scheffe's test and were considered to be significant when the p value was less than 0.05.

Results

Calibration of Sensors

The NO sensor was shown to be stable and to have a high specificity to NO without responding to any change in oxygen concentrations, infusion of ACh, enalapril or losartan, or mixing solution (data not shown). The mean peak response to NO concentration was 343±15 pA/nmol/L among the seven sensors in the present study. This value was comparable to the values obtained with the original sensor (*20*).

Hemodynamic Data after 8-Week Treatment

During the 8-week treatment, the mean arterial pressure (MAP), heart rate and body weight were compared among the experimental groups of hyperlipidemic rabbits as shown in Table 1. MAP was slightly but not significantly lower after treatment with enalapril, losartan, or both compared with the level in the control group. There were no significant differences in heart rate or body weight between any of the drug treatment groups and the control. The TC and TG levels of the four groups were similar at the beginning of the experiment. At 8 weeks, we did not observe any significant differences among groups in either the TC or TG levels (Table 2).

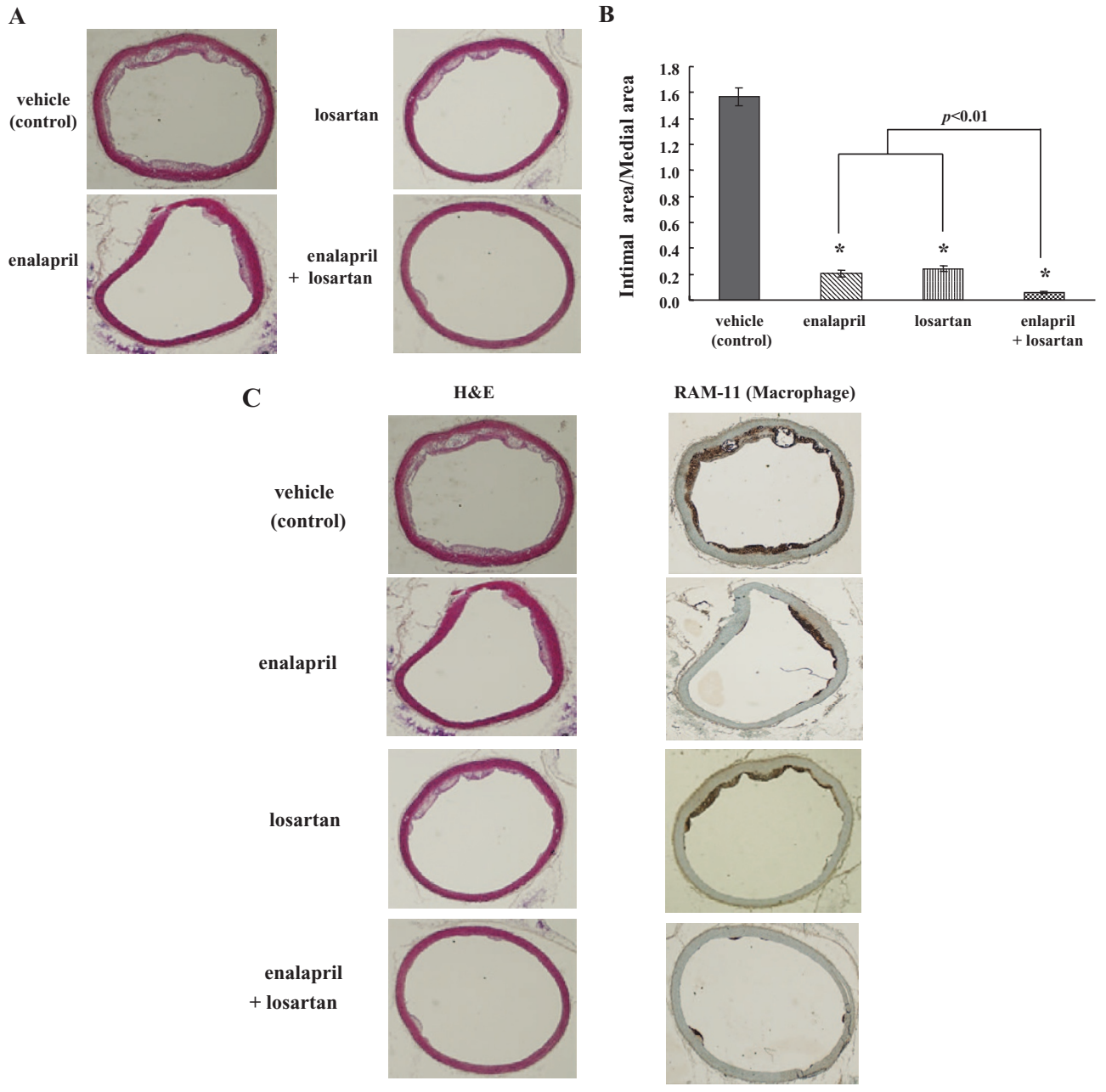

Fig. 4. *A: Photomicrographs of cross-sections of the thoracic aorta. The thoracic aortae sections of WHHL rabbits treated with vehicle (control), enalapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day) were stained with hematoxylin and eosin. B: Ratio of the mean intimal area to the medial area of the thoracic aorta compared in WHHL rabbits treated with vehicle (control), enalapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day). Data are expressed the means ± SEM (n = 6). C: Distribution of macrophage in aortic cross sections among WHHL rabbits treated with vehicle (control), enalapril, losartan, and enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day). Aortic cross sections were stained with monoclonal antibody RAM-11 recognizing macrophage which were compared with sections stained with hematoxylin-eosin.*

ACh-Induced Increase in NO Synthesis after the 8-Week Treatments

Endothelial function was monitored with ACh-induced NO synthesis. Intra-aortic infusion of ACh (20 μg/kg for 5 min) induced an increase in plasma NO concentration, which was significantly greater with all the drug treatments compared with the control ($p < 0.01$). The ACh-induced plasma NO con-

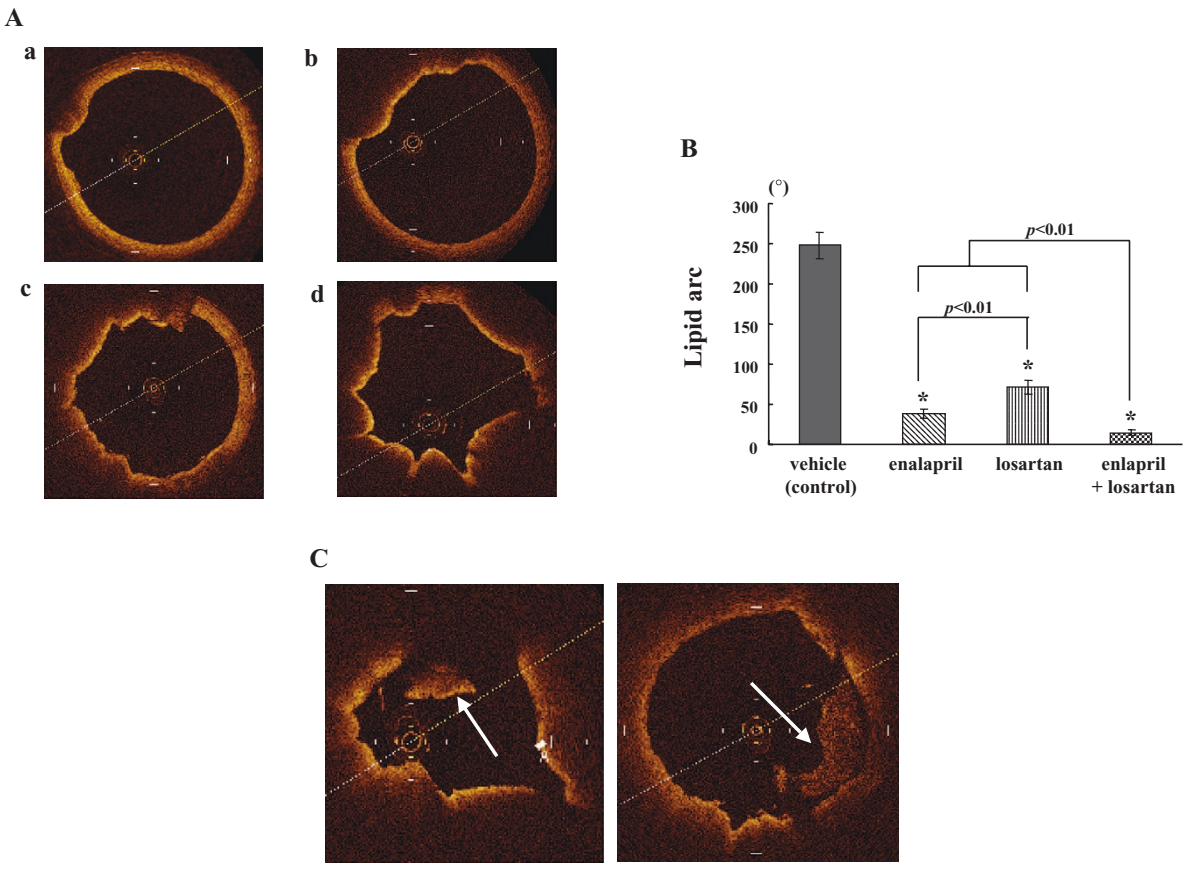

Fig. 5. *OCT images of the thoracic aortae of WHHL rabbits. A: Lipid content was classified into 4 grades according to the number of involved quadrants on the cross-sectional OCT images as follows: 1 quadrant (a), 2 quadrants (b), 3 quadrants (c), and 4 quadrants (d). Lipid-rich plaque (lipid content ≥2 quadrants) in any of the images was only found in vehicle (control) group. B: Degree of lipid arc on the cross-sectional OCT image in involved segments along the thoracic aorta in WHHL rabbits treated with vehicle (control), enalapril (3 mg/kg/day), losartan (30 mg/kg/day), or enalapril-losartan combination (1.5 mg/kg/day and 15 mg/kg/day). OCT identified plaque lesions were quantified as the degree of lipid arc in involved segments along the thoracic aorta with cross-sectional OCT imaging. Data are expressed as the means±SEM (n=6). C: OCT images of thrombus. OCT images demonstrate two types of thrombi, that is, red and white thrombi as previously reported (27). OCT images of red thrombi (a) are characterized as high-backscattering protrusions with signal-free shadowing (arrows). On the other hand, OCT images of white thrombi (b) are visualized as signal-rich, low-backscattering projections (arrows). Both red and white thrombi were exclusively found in the vehicle (control) group.*

centration in the enalapril-treatment group was significantly higher than that in the losartan-treatment group. Combined treatment with enalapril and losartan increased ACh-induced NO by about 9.5 nmol/L, which was significantly greater than the increases in the enalapril and losartan monotherapy-groups (Fig. 1).

Basal NO Synthesis after 8-Week Treatments

The effect of 8-week treatments on local basal NO concentration was evaluated by the decrease in NO concentration in response to infusion of 5 mg/kg of L-NMMA (Fig. 2). All the drug treatments resulted in a significantly higher basal NO concentration compared with that in the control group ($p<0.01$, respectively). In addition, the decrease in the basal plasma NO concentration by L-NMMA infusion was significantly higher in the animals receiving the enalapril-losartan combination (−3.62±0.09 nmol/L) than in those receiving either enalapril (−2.55±0.04 nmol/L, or losartan) or losartan (−1.65±0.08 nmol/L) alone ($p<0.01$, respectively).

Change in Plasma Nitrotyrosine

The plasma nitrotyrosine measured as a surrogate index of the plasma peroxynitrite level was significantly lower in the drug-treated groups than in the control group (Fig. 3). The enalapril-losartan combination produced a significantly lower level of plasma nitrotyrosine (0.54±0.02 pmol/mg protein)

than either enalapril (0.72±0.03 pmol/mg protein) or losartan (0.91±0.03 pmol/mg protein) alone ($p<0.01$), demonstrating the additive effect of enalapril and losartan in reducing peroxynitrite.

Atherosclerotic Plaque Formation

Atherosclerotic plaque formation was assessed in histological sections of the thoracic aortae of WHHL rabbits. Compared to the level in the control group, enalapril or losartan alone markedly attenuated the formation of atherosclerotic plaque. Combined administration of enalapril plus losartan further inhibited the formation compared with enalapril or losartan alone, as shown in Fig. 4A. The atherosclerotic change was quantified by calculating the ratio of the to the medial area in the section, as shown in Fig. 4B. The ratio was smaller by enalapril or losartan alone than in the control animals, and decreased further by combined administration of enalapril and losartan. Immunostaining of the sections with a monoclonal antibody RAM-11 against macrophages (RAM-11) revealed that plaque composition consisted almost exclusively of monocytes/macrophages, irrespective of treatments (Fig. 4C). Furthermore, OCT technology demonstrated that lipid-rich plaque (lipid content ≥2 quadrants) was only present in images of the control group (Fig. 5A). The measured degree of lipid arc along the thoracic aorta was significantly reduced in the enalapril-losartan combination group compared with the groups treated with enalapril or losartan alone (Fig. 5B). In addition, both red and white thrombi were exclusively found in the control group, and were not observed in the treatment groups (Fig. 5C).

ACh-Induced and Basal NO Synthesis after 24 h Treatments

We also examined the short-term effects of enalapril, losartan, and their combination on NO bioavailability in WHHL rabbits ($n=4$ each). In contrast with chronic administration of these drugs, ACh-induced plasma NO concentrations did not differ significantly among 24 h treated groups: vehicle (control), 8.8±0.3 nmol/L; enalapril, 9.5±0.4 nmol/L; losartan, 9.2±0.3 nmol/L; and enalapril-losartan, 9.7±0.4 nmol/L. Similarly, none of the short-term drug treatments yielded a basal NO concentration that was significantly higher than that of the control group. That is, the decrease in the basal plasma NO concentration by L-NMMA did not differ significantly among groups: vehicle (control), −3.35±0.08 nmol/L; enalapril, −3.62±0.12 nmol/L; losartan; −3.42±0.09 nmol/L; and enalapril-losartan combination, −3.84±0.11 nmol/L.

Discussion

In the present study, we have demonstrated for the first time that chronic, but not short-term, administration of either enalapril or losartan increased both the ACh-induced and basal plasma NO concentrations, with enalapril increasing the plasma NO concentration significantly more than losartan. More intriguingly, the combined chronic treatment with enalapril and losartan increased ACh-induced as well as basal plasma NO concentration significantly more than either enalapril or losartan alone.

Several clinical studies have demonstrated that both ACEIs and ARBs improve endothelial function in patients with cardiovascular risk factors or coronary disease (*10–12*). However, in those studies endothelial function was quantified by measuring secondary effects of NO, such as biologically inactive products of NO (nitrite and nitrate) or flow-dependent endothelium-mediated vasodilation. On the other hand, our catheter-type NO sensor has high specificity for NO, and the monitored current reflected changes in the plasma concentration of NO released from the endothelium after ACh and L-NMMA infusions (*7–9*). In the previous reports, Yagi *et al* demonstrated that combined treatment with an ARB, valsartan, and an ACEI, benazepril, had an additive effect on inhibiting neointima formation *via* improvement of nitric oxide production and suppression of oxidative stress in a rat vascular endothelial injury model (*21*). However, accumulation of further evidence in different animal models or using different drugs will be needed before such combination therapy can be applied clinically. In the present study, we examined the effects of the ACEI enalapril, the ARB losartan, or both on NO bioavailability measured with a catheter-type NO sensor, atherosclerotic change, and plasma peroxynitrite level in WHHL rabbits, which develop hyperlipidemia without dietary intervention. In addition, Yagi *et al*. measured biologically inactive products of NOx (nitrite and nitrate) as an index of NO production. This type of evaluation is complicated by the fact that many stimuli perturb the release of NO and its metabolites (*22*). Furthermore, it remains unknown whether endothelial NOS, inducible NOS, or both caused the elevation of the NO end product (*23*). For all of these reasons, the present study provides novel findings distinct from those of the previous study (*21*).

In this study, the effect of the ACEI enalapril and the ARB losartan on NO bioavailability was examined. We have shown for the first time that co-treatment with an ACEI and an ARB significantly increased the ACh-induced as well as the basal plasma NO concentration compared with either ACEI or ARB alone. ACE inhibition may improve NO bioavailability by increasing bradykinin-dependent NO production, and reducing vascular oxidant stress to prevent inactivation of endothelial NO by superoxide (*24*). AT_1 receptor activation by Ang II stimulates NADPH oxidase, resulting in the generation of reactive oxygen species in vascular cells and eventually, endothelial dysfunction (*25*). Therefore, ACEIs improve endothelial function by inhibiting the production of Ang II and ARBs improve endothelial function by blocking the activity of AT_1 receptors. In the present study, the ACEI caused a greater increase in NO production than the ARB in WHHL rabbits. Although ACEIs and ARBs inhibit

the renin-angiotensin system *via* different mechanisms, the present results raised the possibility that ACE inhibition might have a superior effect on NO bioavailability compared with ARB administration in WHHL rabbits. It is believed that, because ARBs block the AT_1 receptor, Ang II is increased by a feedback mechanism, which in turn stimulates the Ang II type 2 (AT_2) receptor, resulting in an increase of NO production (*26*). On the other hand, the improvement of NO production by an ACEI may suppress Ang II production. One possible reason for the different effects of ACEIs and ARBs on NO bioavailability might that the two classes of agents utilize different mechanisms for inhibiting the renin-angiotensin system. Another possibility is that inhibition of kininase II by ACEI decreases the degradation of bradykinin, and the higher level of bradykinin increases local NO production. There is now increasing experimental evidence, however, that ARBs may lead to bradykinin-dependent release of NO from the endothelium, an effect mediated by increased AT_2-receptor stimulation (*27–29*). In the present study, we could not clarify by the ACEI had a more pronounced effect on NO bioavailability than the ARB in WHHL rabbits, because we could not examine whether the ability of the ARB to stimulate AT_2 receptors was directly related to the improvement of endothelial dysfunction as well as atherosclerotic change in WHHL rabbits. Further studies will be needed on this important subjects.

Recent evidence suggests that the interplay between hypercholesterolemia and hypertension acts through the renin-angiotensin system to produce reactive oxygen species. Peroxynitrite ($ONOO^-$) is an important mediator of the oxidation of low-density lipoprotein, emphasizing its pro-atherogenic role (*30*). Furthermore, both superoxide (O_2^-) and $ONOO^-$ have been demonstrated to oxidize BH_4, a critical endothelial NO synthase (eNOS) cofactor, leading to eNOS "uncoupling" (*31*). An uncoupled eNOS produces O_2^- rather than NO. Dysfunction of eNOS has been shown to accelerate atherosclerotic lesion formation in mice (*32*), whereas overexpression of eNOS in mice with hypercholesterolemia resulted in increased eNOS–derived O_2^- production and promotion of atherogenesis (*33*). In the present work, histological as well as OCT studies demonstrated that both the ACEI and the ARB reduced the plaque area. More importantly, the combination treatment with both agents dramatically reduced the plaque area accompanied with marked suppression of the production of plasma peroxynitrite. Kim *et al*. have demonstrated that combination treatment with temocapril (an ACEI) and CS-866 (an ARB) prevented vascular smooth muscle cell proliferation in the intima to a greater extent than monotherapy in a rat balloon-injury model. They also demonstrated that L-NMMA, an NO synthase inhibitor, significantly attenuated the diminished intimal hyperplasia induced by combined administration of temocapril and CS-866 (*34*). Taking these findings together, one may speculate that the anti-atherosclerotic effect of the combination therapy in the present study was derived from the inhibition of NO synthesis. However, this study did not investigate whether the inhibition of NO synthase could reverse the beneficial effects of the combination therapy on atherosclerotic change. Therefore, we could not conclude that the anti-atherosclerotic effect of the combination therapy was derived from the inhibition of NO synthase in WHHL rabbits. Further studies will be needed on this important subject.

The unique capability of OCT to quantify the size of lipid-rich plaque and thickness of the cap had been clearly confirmed by several groups (*18*, *35*, *36*). Because OCT systems allowed screening of long arterial segments, it is possible to thoroughly examine the effects of drugs on atherosclerotic change along the thoracic aorta using this method. Furthermore, OCT can allow us not only to estimate plaque morphology but also to identify thrombus (*37*). Indeed, the present study has demonstrated that both red and white thrombi were found only in the control (no-treatment) group.

The pharmacological differences in the mechanisms by which ARBs and ACEIs suppress the renin-angiotensin system may have important clinical implications. In terms of heart failure, the combined treatment with both classes of drugs has been shown to have clinical efficacy. In the Valsartan Heart Failure Trial (Val-HeFT), in which some 93% of patients received ACEI, valsartan 160 mg/day reduced hospital admission for congestive heart failure by 27.5% compared with placebo (*38*). On the other hand, although the present study has demonstrated that combined treatment with an ACEI and an ARB had additive effects on NO bioavailability in addition to including atherosclerotic change in WHHL rabbits, there is currently no clinical evidence that combination therapy with an ACEI and an ARB significantly ameliorates atherosclerosis. Some assessments of the relative merits of treatment with an ACEI, an ARB, and or both will be provided by the Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) Programme (*39*).

Accumulating data suggest that improved endothelial function may make an important contribution to the beneficial effects of ACEIs and ARBs on cardiovascular events. In order to investigate the details of this relation, and particularly the relationship between increased NO bioavailability and reduced atherosclerosis, a catheter-type NO sensor is potentially useful as a tool. Pharmacological modulation of NO can be studied in details by measuring the basal and ACh-induced NO production in experimental animal models. The NO sensor may also be applied to clinically diagnose endothelial function, *i.e.*, reduced endothelium-derived NO bioavailability in patients with the cardiovascular disease. Future therapeutic strategies in human atherosclerosis may focus on preventing the development of endothelial dysfunction, as assessed by the NO sensor.

Conclusion

We have demonstrated that co-administration of an ACEI and

an ARB had beneficial effects on NO bioavailability assessed by using a catheter-type NO sensor, as well as on vascular remodeling evaluated by histology and OCT in WHHL rabbits.

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