Tissue Angiotensin-Converting-Enzyme (ACE) Deficiency Leads to a Reduction in Oxidative Stress and in Atherosclerosis

Studies in ACE-Knockout Mice Type 2

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Background—Angiotensin II, produced by angiotensin-converting-enzyme (ACE), enhances oxidative stress and atherogenesis. In this study, we analyzed whether tissue ACE deficiency in ACE-knockout mice type-2 would affect their oxidative status. Moreover, by crossbreeding the ACE-knockout mice with atherosclerotic apolipoprotein E (apo E)–deficient (E0) mice, we questioned whether tissue ACE deficiency affects atherogenesis.

Methods and Results—ACE-deficient mice type-2 (ACE/H11001/H11002) exhibited reduced serum lipid peroxidation compared with ACE/H11001/H11001 mice. Peritoneal macrophages from ACE/H11001/H11002 mice demonstrated lower oxidative status, as exhibited by decreases of 47%, 33%, 56%, and 51%, in their lipid peroxides, superoxide release, dichlorofluorescein fluorescence, and LDL oxidation, respectively, compared with ACE/H11001/H11001 mice. ACE/H11001/H11002 mice crossbred with E0 mice, resulting in atherosclerotic mice heterozygous for ACE (ACE/H11001/H11002/E0 mice), exhibited reduced lipid peroxidation, increased paraoxonase activity, and lower macrophage LDL oxidation compared with E0 and ACE/H11001/H11001/E0 mice. ACE/H11001/H11002/E0 mice also exhibited reduced NADPH-induced aortic superoxide ion production by 52% and a reduction of 43% in their atherosclerotic lesion size compared with E0 mice. Finally, 2 animals genotyped as homozygous-knockout for both ACE and APOE genes (ACE/E0), exhibited a striking reduction of 86% in their atherosclerotic lesion area compared with E0 mice.

Conclusions—Reduction of tissue ACE with the ACE-knockout mouse type-2 model inhibited oxidative stress and atherogenesis. (Arterioscler Thromb Vasc Biol. 2003;23:2090-2096.)

Key Words: angiotensin-converting enzyme ▪ atherosclerosis ▪ knockout mice ▪ lipoproteins

Oxidative stress is involved in atherogenesis and is associated with lipid peroxidation in lipoproteins and arterial macrophages. Under oxidative stress, macrophages generate reactive oxygen species, leading to LDL oxidation, increased oxidized-LDL uptake, and foam cell formation. Serum paraoxonase (PON1), an HDL-associated esterase, protects lipoproteins and atherosclerotic lesions against oxidative stress. PON1 is inactivated by lipid peroxides, and serum PON1 activity is reduced in atherosclerotic patients.

Angiotensin II (Ang II), produced by the action of angiotensin-converting-enzyme (ACE) on Ang I, accelerates atherogenesis by stimulating smooth muscle cell proliferation, platelet activation, and cholesterol accumulation in arterial macrophages. Ang II activates macrophage NADPH oxidase, leading to increased macrophage lipid peroxidation and LDL oxidation.

ACE is one of the candidate genes of the renin-angiotensin system (RAS) that might be involved in atherogenesis. Beside its localization and activity in the circulation, ACE is localized in macrophages and smooth muscle cells of human atherosclerotic plaques. Tissue ACE might participate in the local RAS that functions independently of the circulating RAS. Use of ACE inhibitors or knockout of the ACE gene is supposed to lower Ang II levels and reduce Ang II proatherogenic properties.

ACE inhibitors attenuate atherogenesis in hypercholesterolemic animals by several mechanisms, including blood pressure reduction, bradykinin level increases, LDL oxidation attenuation, and macrophage foam cell formation reduction. The Heart Outcomes Prevention Evaluation (HOPE) study showed that the ACE inhibitor ramipril reduces mortality, myocardial infarction, and stroke in patients at high risk for cardiovascular events.

Several models of ACE-knockout mice have been generated to study the physiologic role of ACE in blood pressure, renal function, and the cardiovascular system. One of the

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2090
these models, the ACE-knockout mouse type-2 (ACE/2 mouse; ACE<sup>−/−</sup>),<sup>20</sup> expresses a truncated form of somatic ACE and thus, exhibits reduced plasma ACE activity but completely lacks tissue ACE. In this study, we analyzed whether tissue ACE deficiency in the ACE<sup>−/−</sup> mouse model would affect oxidative stress. Moreover, by crossing ACE<sup>−/−</sup> mice with atherosclerotic, apolipoprotein E (apo E)<sup>−/−</sup> mice, we questioned whether tissue ACE deficiency affects atherogenesis.

**Methods**

**Mice**

E<sup>0</sup> Mice

Apo E−deficient (E<sup>0</sup>) mice on a C57BL/6J background (provided by Prof Jan Breslow, Rockefeller University, New York, NY) are widely used as an animal model for atherosclerosis because they develop severe hypercholesterolemia on a chow diet.<sup>21</sup>

ACE<sup>+/−</sup> Mice

ACE-knockout mice type-2 (ACE/2 mice, or ACE<sup>−/−</sup>; on a C57BL/6J background) were provided by Prof Ken Bernstein (Department of Pathology, Emory University, Atlanta, Ga).<sup>20</sup> ACE<sup>+/−</sup> mice were backcrossed for 4 generations and were bred as heterozygous (ACE<sup>+/−</sup>) mice, because mice homozygous for the ACE gene are almost sterile. Therefore, mice were genotyped by polymerase chain reaction (PCR) of tail DNA, as previously described.<sup>19,20</sup> DNA was isolated from mouse tails by proteinase K treatment. PCR typing was performed with a set of 3 primers: (1) KP4-1, 5′-GGTTGTTCAAGACTACAATCTGACC-3′; (2) ACE0.383, 5′-AGCTTACGGGCTCTGTTCCGTGC-3′; and (3) ACE0.225, 5′-CAGACGAGCTCGGGATCTGGGTTGTCTGGT-3′.

The PCR conditions were as follows: denaturation (94°C, 30 seconds), annealing (58°C, 1 minute, for 40 cycles), and extension (72°C, 1 minute). PCR products were analyzed by 2% agarose gel. The wild-type ACE locus yields a 215-bp fragment, whereas the modified locus (in the ACE<sup>−/−</sup> mice) generates a 287-bp product. DNA isolated from ACE<sup>−/−</sup> mice exhibits both the 215-bp and the 287-bp PCR products.

**Crossbreeding of ACE<sup>+/−</sup> Mice With E<sup>0</sup> Mice**

Female mice heterozygous for the ACE gene (ie, ACE<sup>+/−</sup>) were mated with male E<sup>0</sup> mice, generating F<sub>1</sub> offspring that were either heterozygous or of the wild type for the ACE gene and heterozygous for the apo E gene. F<sub>1</sub> mice that were identified as ACE<sup>+/−</sup>/apoE<sup>−/−</sup> were then mated between themselves to produce F<sub>2</sub> offspring. F<sub>2</sub> offspring that were heterozygous for the ACE gene and homozygous for the apo E gene were used for this study (ACE<sup>+/−</sup>/apoE<sup>−/−</sup>). From the F<sub>2</sub> offspring, only 2 mice were identified as double knockouts for both the ACE gene and the apo E gene (ACE<sup>−/−</sup>/apoE<sup>−/−</sup>), and they were analyzed for their atherosclerotic lesion area. All of the mice used in this study were male. Controls for the ACE<sup>−/−</sup> mice were littermate, age-matched ACE<sup>−/−</sup> mice. Controls for ACE<sup>−/−</sup>/apoE<sup>−/−</sup> and ACE<sup>−/−</sup>/apoE<sup>0</sup> mice were age-matched E<sup>0</sup> mice, as well as littermate ACE<sup>+/−</sup>/apoE<sup>0</sup> mice.

**Mouse Blood Pressure Measurements**

Computerized blood pressure measurements were performed with a tail-cuff apparatus (5 measurements per mouse) with the use of a monitoring device (model 229 ITT, NIBP System) and appropriate computer software (model 31 ITT, Life Science Instruments).

**Serum Analyses**

**Serum ACE Activity and Lipid Profile**

Blood was collected from the mouse retroorbital plexus under ether anesthesia. Serum ACE activity,<sup>22</sup> serum cholesterol, and triglycerides were determined with commercial kits (Sigma) with a UV microplate reader (PowerWave<sub>®</sub>, Biotech).

**Serum Lipid Peroxidation and PON1 Activity**

Serum samples were incubated (AAPH, Wako Chemical Industries) for 2 hours at 37°C. Serum lipid peroxide content was determined by the thiobarbituric acid–reactive substance (TBARS) assay<sup>23</sup> and the lipid peroxide (PD) assay.<sup>24</sup> Serum PON1 activity was determined spectrophotometrically at 270 nm by its arylesterase activity, with phenylacetate as a substrate. The extinction coefficient at 270 nm for the reaction is 1310 (mol/L)<sup>−1</sup> cm<sup>−1</sup>.<sup>25</sup>

**Mouse Peritoneal Macrophages**

**MPM Isolation**

Mouse peritoneal macrophages (MPMs) were harvested from the peritoneum 4 days after intraperitoneal injection of thioglycolate (3 mL of 24 g/L). In some experiments, ACE<sup>−/−</sup> mice and ACE<sup>+/−</sup> mice were injected with Ang II (0.1 mL of 10<sup>−5</sup> mol/L) for 7 days before cell harvesting.

**MMP ACE Activity Determination**

MPMs plated into 96-well plates (10<sup>3</sup> cells/well) were analyzed in situ for their ACE activity.<sup>22</sup> Results are expressed as milliliters per milligram cell protein.

**MMP Lipid Peroxidation Determination**

MPM PD was assayed in MPM sonicates.<sup>26</sup>

**MMP Release of Superoxide Ions**

Cells (10<sup>5</sup> cells/well) incubated in Hanks’ balanced salt solution containing acetylsalicylic acid (80 µmol/L) were stimulated by phorbol myristate acetate (0.5 µg/mL) for 30 minutes. Superoxide dismutase (30 mg/L) was added to control samples. Superoxide release levels were expressed as nanomoles superoxide per milligram cell protein, with an extinction coefficient of 550 nm of 21 (mmol/L)<sup>−1</sup> cm<sup>−1</sup>.

**Flow Cytometric Assay of DCFH-DA Oxidation**

MPM peroxide levels were determined by flow cytometric assay with dichlorofluorescein diacetate (DCFH-DA).<sup>28</sup> For additional details, see the online Methods section at http://atvb.ahajournals.org.)

**LDL Oxidation by MPMs**

MPMs were incubated with human LDL, and LDL oxidation was measured in the medium by the TBARS assay.<sup>23</sup> For additional details, see the online Methods section at http://atvb.ahajournals.org.)

**NADPH-Mediated Detection of O<sub>2</sub>− Production in Vascular Tissue by Lucigenin-Derived Chemiluminescence**

Vascular O<sub>2</sub>− formation was measured by the lucigenin-enhanced chemiluminescence assay. Mouse thoracic aortas were removed, placed into prewarmed Krebs-Henseleit/HEPES buffer, and cut into 3-mm ring segments. Segments were transferred to a white 96-well microplate containing Krebs-Henseleit/HEPES buffer (100 µL) with lucigenin (10 µmol/L; this low concentration prevents auto-oxidation<sup>29,30</sup>). NADPH (100 µmol/L) was added to each well after 10 minutes. Chemiluminescence was recorded every 2 minutes in a microplate luminometer at 37°C (Lucy-1, Rosys Anthos). The vessels were dried (24 hours at 90°C) and weighed. Results are expressed as counts per minute per milligram dry tissue.

**Histopathology of Aortic Atherosclerotic Lesions**

See the online Methods section at http://atvb.ahajournals.org.)

**Statistics**

Student’s t test (2 tailed) was performed for comparing 2 arrays of data, and ANOVA was used when >2 groups were being compared. For statistical analysis of parameters without a gaussian distribution, such as triglycerides, the values were logarithmically transformed to create a gaussian distribution. Results are expressed as mean±SD except for atherosclerotic lesion area measurements, which are given as mean±SEM.
Results

Oxidative Stress in ACE<sup>+/−</sup> Mice

Serum Lipid Peroxidation in ACE<sup>+/−</sup> Mice

ACE<sup>+/−</sup> mice exhibited almost similar systolic and diastolic blood pressures as well as serum lipid profiles (as determined by serum cholesterol or triglyceride levels) compared with wild-type ACE<sup>+/+</sup> mice (see online Table I at http://atvb.ahajournals.org). Serum ACE activity in ACE<sup>+/−</sup> mice was lower by 37% compared with ACE<sup>+/+</sup> mice (Figure 1A).

The susceptibility to oxidation of sera isolated from ACE<sup>+/−</sup> mice determined by the TBARS and PD assays was significantly lower by 29% (Figure 1B) and by 24%; from 842±86 nmol PD/mL serum in ACE<sup>+/+</sup> to 656±70 nmol PD/mL serum from ACE<sup>+/−</sup> mice, respectively, compared with values in wild-type, ACE<sup>+/+</sup> mice sera.

Serum PON1 activity, which was previously shown to be inactivated by oxidative stress, was significantly higher in ACE<sup>+/−</sup> mice, by 44%, compared with that in wild-type, ACE<sup>+/+</sup> mice: from 59.5±14.7 U/mL in serum from ACE<sup>+/+</sup> mice to 85.9±13.6 U/mL in serum from ACE<sup>+/+</sup> mice. An inverse correlation (r²=0.51) was obtained after plotting mouse serum ACE activity levels against serum PON1 activity values obtained from both ACE<sup>+/+</sup> and ACE<sup>+/−</sup> mice (Figure 1C).

MPM Oxidative Stress in ACE<sup>+/−</sup> Mice: Effect of Ang II

Macrophage ACE activity in ACE<sup>+/−</sup> mice was significantly lower, by 30%, compared with ACE activity in ACE<sup>+/+</sup> mice (Table 1). We next questioned whether the reduced serum oxidative stress in ACE<sup>+/−</sup> mice might also be expressed in cells such as macrophages. MPMs from ACE<sup>+/−</sup> mice indeed contained significantly lower levels of lipid peroxides, superoxide release, and DCF mean fluorescence by 47%, 33%, and 56%, respectively, compared with MPMs from ACE<sup>+/+</sup> mice (Table 1). Because oxidation of LDL by arterial macrophages takes place during early atherogenesis and oxidized macrophages oxidize LDL at an enhanced rate, we analyzed the ability of MPMs derived from ACE<sup>+/+</sup> mice to oxidize LDL. LDL oxidation by MPMs from ACE<sup>+/−</sup> mice was indeed significantly lower, by 51%, compared with LDL oxidation by MPMs from ACE<sup>+/+</sup> mice (Table 1).

To assess whether the inhibitory effect of ACE deficiency on macrophage proatherogenic properties could be reversed by Ang II, ACE<sup>+/+</sup> mice were injected with Ang II (0.1 mL of 10<sup>−5</sup>mol/L per mouse per day) for 7 days. Ang II injection into ACE<sup>+/+</sup> mice significantly increased their macrophage lipid peroxidation by 129% compared with untreated ACE<sup>+/+</sup> mice. Macrophage lipid peroxide levels in cells from Ang II–treated ACE<sup>+/+</sup> mice were even higher, by 71%, than those found in ACE<sup>+/+</sup> mice (Table 2). Similarly, Ang II injection into ACE<sup>+/−</sup> mice significantly increased their macrophage-mediated LDL oxidation by 100% compared with untreated ACE<sup>+/−</sup> mice, to levels similar to those observed in ACE<sup>+/+</sup> mice (Table 2).
TABLE 2. The Effect of Ang-II Injections to ACE-Deficient Mice on Macrophage Lipid Peroxidation and Macrophage-Mediated LDL Oxidation

<table>
<thead>
<tr>
<th></th>
<th>ACE+/+</th>
<th>ACE+/−</th>
<th>ACE−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage PD, nmol PD/mg cell protein</td>
<td>30.7±4.3</td>
<td>22.9±7.2*</td>
<td>52.6±5.1†</td>
</tr>
<tr>
<td>Macrophage-mediated LDL oxidation, nmol MDA/mg LDL protein</td>
<td>58.6±0.48</td>
<td>31.9±3.5*</td>
<td>63.8±6.2†</td>
</tr>
</tbody>
</table>

All values represent mean±SD. n=3 in each group.
*P<0.01 vs ACE+/+; †P<0.01 vs ACE+/−.

Blood Pressure and Lipid Profile in ACE+/−/E0 Mice

ACE+/− mice were crossed with E0 mice to create offspring that were ACE+/−/E0 mice. ACE+/−/E0 mice exhibited almost similar systolic and diastolic blood pressures as well as similar serum lipid profiles (as determined by their serum cholesterol and triglyceride concentrations) compared with age-matched E0 mice (see online Table II at http://atvb.ahajournals.org).

Oxidative Stress in Sera and Macrophages From ACE+/−/E0 Mice

Serum ACE activity in ACE+/−/E0 mice was lower by 24% compared with that in ACE+/+ mice (Figure 2A). Susceptibility to oxidation of sera isolated from ACE+/−/E0 mice, as determined by the TBARS and PD assays, was significantly lower by 41% (Figure 2B) and by 45% (from 2243±2.70 nmol PD/mL serum in E0 mice to 1252±126 nmol PD/mL serum in ACE+/−/E0 mice), respectively, compared with age-matched, control E0 mouse sera. In parallel, serum PON1 activity in ACE+/−/E0 mice was significantly higher by 29% compared with age-matched E0 mice (Figure 2C).

We next studied oxidative stress in mouse macrophages. MPMs derived from ACE+/−/E0 mice were analyzed for their ACE activity and exhibited a decrease in cellular ACE activity, by 33%, compared with ACE activity measured in MPMs from E0 mice (Figure 3D). Moreover, the ability of macrophages from ACE+/−/E0 mice to oxidize LDL was decreased by 27% compared with that in E0 mice, further illustrating a reduction in the oxidative status of macrophages isolated from ACE+/−/E0 mice compared with those from E0 mice (Figure 3E).

ACE+/−/E0 mice that were littermates of the ACE+/−/E0 mice were analyzed for their ACE activity and oxidative stress. ACE+/−/E0 mice (n=8) exhibited a serum ACE activity of 175±9 U/L, serum lipid peroxidation of 80.8±6 nmol malondialdehyde (MDA)/mL, a serum PON1 activity of 45.4±5 U/mL, and macrophage ACE activity of 38±3 mU/mg cell protein, similar to those values obtained in the original E0 mice (Figure 2). Moreover, LDL oxidation by macrophages from ACE+/−/E0 mice was similar to that obtained in macrophages from original E0 mice (data not shown).

Effect of ACE Deficiency on Vascular Superoxide Ion Production and Atherosclerotic Lesion Size and Morphology

To determine whether ACE deficiency affected aortic superoxide production, aortic segments were isolated from ACE+/−/E0 and E0 mice and analyzed for their NADPH-induced superoxide ion production. ACE+/−/E0 mice exhibited significantly decreased superoxide production by up to 52% compared with control E0 mice (Figure 3A). We next examined whether reduced oxidative stress in sera, macrophages, and aortic segments from ACE+/−/E0 mice was associated with a reduction in atherosclerotic lesion development. ACE+/−/E0 mice exhibited a significant decrease of 43% in their aortic atherosclerotic lesion area compared with age-matched E0 mice (Figure 3B). ACE+/−/E0 mice (n=8) that were littermates of the ACE+/−/E0 mice exhibited an atherosclerotic lesion area of 40.032±1261 µm², similar to that obtained in original E0 mice (Figure 3). Most...
important, 2 animals that were genotyped as double knockouts for both the ACE and APOE genes (ACE<sup>+/H11002</sup>/E<sub>0</sub>) exhibited a striking reduction of 86% in their atherosclerotic lesion area compared with age-matched E<sub>0</sub> mice. ACE<sup>+/H11002</sup>/E<sub>0</sub> mice exhibited an atherosclerotic lesion area of 57 962 ± 7741 m<sup>2</sup> compared with 42 032 ± 17250 and 40 032 ± 1261 m<sup>2</sup> in age-matched E<sub>0</sub> and ACE<sup>+/H11001</sup>/E<sub>0</sub> mice, respectively. Aortas from ACE<sup>+/H11001</sup>/E<sub>0</sub> mice showed less advanced lesions than did those from E<sub>0</sub> mice, as illustrated by a reduction in cholesterol crystals and calcification (Figure 3C and 3D), and these features were even further reduced in the ACE<sup>+/H11002</sup>/E<sub>0</sub> mice (Figure 3E).

**Discussion**

This study demonstrates that knockout of the ACE gene in mice caused a decrease in lipid peroxide content in their sera and peritoneal macrophages. Injection of Ang II into ACE<sup>+/</sup>/E<sub>0</sub> mice increased macrophage lipid peroxidation and the macrophages’ ability to oxidize LDL to levels similar to those found in ACE<sup>+/</sup>/E<sub>0</sub> mice. Crossbreeding of E<sub>0</sub> mice with the ACE<sup>+/</sup>/E<sub>0</sub> mice resulted in offspring homozygous for APOE and heterozygous for ACE, and these animals exhibited decreased atherogenesis compared with E<sub>0</sub> mice. These mice demonstrated significant reduced serum, macrophage, and aortic oxidative stress compared with E<sub>0</sub> mice. Most important, reduced oxidative stress in ACE<sup>+/</sup>/E<sub>0</sub> mice was associated with reduced atherosclerotic lesion area compared with age-matched, E<sub>0</sub> mice.

In this study, we used a model of knockout mouse, the ACE-knockout mouse type-2, which expresses a truncated form of somatic ACE and thus, exhibits reduced plasma ACE activity but completely lacks tissue ACE. The use of ACE-knockout mice provides additional and important information over the use of ACE inhibitor administration to atherosclerotic mice regarding the role of ACE in atherosclerosis and macrophage oxidative stress. ACE inhibitors possess additional biologic effects not exclusively linked to ACE inhibition and Ang II lowering. Furthermore, the ACE-knockout mouse model is more physiopathologic than is ACE inhibitor therapy; thus, to understand the central role of ACE in atherosclerosis, a model is needed, especially because both circulating ACE and tissue ACE are affected in the ACE-knockout mouse type-2 model, whereas the extent of inhibition of tissue ACE by ACE inhibitors is uncertain.

Most of the ACE enzymatic activity is associated with tissues. The functional significance of circulating ACE versus tissue-bound ACE has provoked interest because lung ACE initiates conversion of Ang I to Ang II. Tissue-bound ACE might participate in the local RAS that functions independently of the circulating RAS. The ACE-deficient mice used in this study were characterized by lack of tissue ACE. Somatic ACE in these animals was truncated and lacked the membrane-spanning “anchor” region and therefore, was found only as a soluble enzyme. Homozygous ACE-knockout mice type-2 have reduced serum ACE activity and completely lack tissue ACE. In addition, because testis
ACE is also affected, male ACE/2 mice are almost sterile. These facts explain the major technical problems in raising a population of homozygous ACE-deficient mice. Breeding is conducted between heterozygous (ACE+/−) animals, and only a few homozygous offspring were obtained when breeding ACE-deficient mice or crossbreeding ACE-deficient mice with E0 mice. Therefore, most of our study was conducted on ACE+/− mice. A substantive reduction in atherosclerotic lesion area observed in ACE+/−/E0 mice (43%) was confirmed in 2 homozygous ACE−/−/E0 animals. In these mice, reduction in atherosclerotic lesion area was as high as 86% compared with age-matched E0 mice. A dose-dependent reduction in the ACE gene thus led to a dose-dependent reduction in ACE−/−/E0 mice atherosclerotic lesion size.

In a previous study,34 ACE-deficient mice were crossbred with E0 mice and analyzed for their atherosclerotic lesion size and morphology. ACE deficiency in E0 mice in the aforementioned study, as opposed to our study, did not influence the size or complexity of the atherosclerotic lesions. However, there are some major differences between the study of Krege et al34 and our study. First, ACE-deficient mice from both studies were developed in different laboratories, and it is likely that their genotype and phenotype are different. Second, the population of ACE-deficient/apo E−/− mice used in the Krege study34 consisted of mice heterozygous for the APOE gene. In addition, the apo E−/− mice used in the Krege study were subjected to a high-cholesterol diet to develop atherosclerosis. However, in our study, E0 mice develop atherosclerotic lesions on a normal chow diet. The controls for ACE+/−/E0 and ACE−/−/E0 mice were E0 from the original strain, as well as littermate ACE−/−/E0, to ensure that changes in atherosclerosis did not result from background differences. It is important to note that apo E−/− mice exhibited higher levels of serum ACE activity than did control mice. The underlying mechanism for this phenomenon might be related to increased oxidative stress or increased serum cholesterol concentration in E0 mice versus control mice.

In this study, Ang II injections to ACE-deficient mice restored their oxidative status and macrophage atherogenic properties to levels observed in wild-type animals (ACE+/+ mice). In this experiment, Ang II effects might be the result of altered cellular signaling pathways in the in vivo system rather than just the presence of Ang II in the ex vivo system. We have also previously shown that ACE inhibitor supplementation to atherosclerotic E0 mice significantly reduced their sera and macrophages oxidative status.14-16 In this study, reduction of ACE activity was achieved by partial knock-out of the ACE gene. Under both cases (ACE inhibitors and ACE-knockout mice), ACE activity reduction was associated with reduced serum and macrophage oxidative stress and reduced atherogenesis. Knockout of the ACE gene could also result in reduced macrophage oxidative stress through an Ang II-independent mechanism, such as by increasing bradykinin levels or increasing NO production.

In this study, we searched for some possible mechanisms related to the reduced atherosclerosis observed in ACE-deficient mice. Macrophage foam cell formation is an early event of atherogenesis, and oxidative stress stimulated the macrophage conversion to foam cells.2 We therefore analyzed lipid peroxidation in ACE-deficient mouse sera and MPMs and compared them with those from ACE wild-type mice. Reduced oxidative stress in ACE-deficient mice was demonstrated in their sera by determination of serum susceptibility to lipid peroxidation and by analysis of serum PON1 activity. Serum susceptibility to lipid peroxidation indicates the presence of preformed lipid peroxides in serum lipoproteins,35 which is related to lipoprotein susceptibility to oxidation. Reduction of ACE thus probably reduced the level of preformed lipid peroxides in lipoproteins.

PON1 activity measurement can also reflect the serum oxidative status, because PON1 is inactivated by lipid peroxides, and a decreased serum PON1 activity was shown under oxidative stress.3 A reduction in oxidative stress in ACE-deficient mice could inhibit PON1 inactivation, leading to increased serum PON1 activity. Furthermore, activation of PON1 could contribute to oxidative stress reduction by its ability to hydrolyze specific accumulated lipid peroxides in serum and arterial cells.

Reduced lipid peroxidation, as measured by several parameters, such as macrophage lipid peroxide content, macrophage superoxide release, and macrophage DCF fluorescence, was observed in MPMs from ACE-deficient mice and was associated with reduced macrophage ACE activity and decreased macrophage ability to oxidize LDL, compared with wild-type mice. Because MPMs mimic arterial macrophage properties,5 the foregoing data suggest that arterial macrophages from ACE-deficient mice possess a decreased ability to oxidize LDL, probably by reduction of NADPH oxidase activity, as shown by reduced aortic superoxide ion production induced by NADPH. As a result of reduction in oxidized LDL formation, the conversion of macrophages to foam cells was reduced and progression of atherosclerotic lesions was inhibited. ACE mRNA and protein have been previously detected in macrophage foam cells present at the shoulder regions of human atherosclerotic lesions,12 and enhanced expression of ACE was associated with progression of coronary atherosclerosis.36 Taken together, the results of this study clearly show that ACE is involved in the buildup of the atherosclerotic lesion.

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