Retardation of Atherosclerosis by Overexpression of Catalase or Both Cu/Zn-Superoxide Dismutase and Catalase in Mice Lacking Apolipoprotein E

Hong Yang, L. Jackson Roberts, MingJian Shi, LiChun Zhou, Billy R. Ballard, Arlan Richardson, ZhongMao Guo

Abstract—Oxidative stress has been suggested to potentiate atherogenesis. However, studies that have investigated the effect of antioxidants on atherosclerosis showed inconsistent results, i.e., atherosclerosis was either retarded or not changed by dietary antioxidants. This report directly examined the effect of overexpressing Cu/Zn-superoxide dismutase (Cu/Zn-SOD) and/or catalase on atherosclerosis and lipid peroxidation in mice lacking apolipoprotein E (ApoE⁻/⁻). Based on lipid staining of the en face of the aorta tree and the serial sections of the proximal aorta, ApoE⁻/⁻ mice overexpressing catalase or both Cu/Zn-SOD and catalase had smaller and relatively early stages of atherosclerotic lesions (e.g., foam cells and free lipids) when compared with ApoE⁻/⁻ mice, who developed more advanced lesions (e.g., fibrous caps and acellular areas). In addition, the retarded development of atherosclerosis was correlated with a reduced F₂-isoprostanes in the plasma and aortas in ApoE⁻/⁻ mice overexpressing catalase or both Cu/Zn-SOD and catalase. In contrast, the levels of F₂-isoprostanes and atherosclerosis in the ApoE⁻/⁻ mice overexpressing Cu/Zn-SOD alone were comparable to ApoE⁻/⁻ control mice. These observations implied that endogenously produced hydrogen peroxide, but not superoxide anions, contributed to the formation of oxidized lipids and the development of atherosclerosis in ApoE⁻/⁻ mice. (Circ Res. 2004;95:000-000.)

Key Words: Cu/Zn-superoxide dismutase ■ catalase ■ atherosclerosis ■ F₂-isoprostanes

Accumulation of oxidized lipids in the arterial wall has been implicated to give rise to atherosclerosis (see reviews1,2). This theory is supported by the findings that the development of atherosclerosis is accompanied by an accumulation of oxidized lipids/lipoproteins in the arterial wall, and that oxidized lipids in vitro induce a number of events putatively involved in atherogenesis such as increasing adherence of inflammatory cells to endothelial cells and inducing vascular cell death and proliferation.1,2 Increasing the antioxidant defense against lipid peroxidation therefore has been proposed to retard the development of atherosclerosis. However, studies that have investigated the effect of dietary antioxidants on atherosclerosis showed inconsistent results, i.e., atherosclerosis was either retarded3-5 or not changed6-8 by antioxidants such as probucol, vitamin E, and vitamin C in humans and animal models. These controversial results are not surprising because there are several limitations in using dietary antioxidants to study the role of oxidative stress in a biological process. For example, some antioxidants, such as vitamin E9 and vitamin C,10 reduce oxidative stress at low concentrations but function as oxidants at higher ones. In addition, some antioxidants have other biological effects besides radical scavenging activity. For example, probucol can lower plasma lipid, which is known to inhibit the development of atherosclerosis. Probucol also has been shown to stimulate the expression of fibrinogen that could promote atherosclerosis.11 Therefore, it is difficult to test the role of oxidative stress in atherosclerosis using dietary antioxidants.

Evidence from in vitro studies suggests that the endogenously produced reactive oxygen species (ROS) are, at least in part, responsible for the formation of oxidized lipids/lipoproteins. For example, it has been shown that increase in superoxide dismutase (SOD) activity reduces vascular cell-mediated oxidation of low-density lipoprotein (LDL),12-14 suggesting that superoxide (O₂⁻) generated in vascular cells is involved in the oxidation of lipids/lipoproteins.14 However, O₂⁻ hardly diffuse through plasma membrane and does not readily react with most biological molecules15; therefore, it has been suggested that the toxicity attributed to O₂⁻ may be mediated by other radical species derived from O₂⁻. Superoxide anions can be converted to hydrogen peroxide (H₂O₂) in...
cells. H$_2$O$_2$ can readily cross cellular membrane and form hydroxyl radicals through its interaction with reox-active transitional metals. In a previous study, we observed that overexpression of Cu/Zn-SOD or catalase reduced LDL oxidation mediated by mouse aorta segments and smooth muscle cells (SMCs), and that the inhibitory effect resulted from overexpressing catalase was greater than overexpressing Cu/Zn-SOD. These observations support the notion that H$_2$O$_2$, released from vascular cells is involved in the formation of oxidized LDL (oxLDL). It has been suggested that SODs and catalase work in concert to detoxify O$_2$ and develop atherosclerotic lesions under normal chow diet. Our results showed that overexpression of catalase alone or overexpression of Cu/Zn-SOD and catalase in combination significantly reduced the level of F$_2$-isoprostane and atherosclerosis in ApoE$^{-/-}$ mice, whereas overexpression of Cu/Zn-SOD alone did not significantly reduce the level of F$_2$-isoprostane and atherosclerosis in these mice. These observations suggest that endogenously produced H$_2$O$_2$, but not O$_2^-$, is a factor promoting the formation of oxidized lipids and the development of atherosclerosis in ApoE$^{-/-}$ mice. Thus, overexpression of antioxidant enzymes or administration of chemical antioxidants that can scavenge H$_2$O$_2$, would provide a benefit treatment for atherosclerosis.

**Materials and Methods**

**Animals**

Transgenic mice overexpressing Cu/Zn-SOD (hSod1/Tg$^{+}$) or catalase (hCat$^{+}$) were respectively generated by injection of fertilized 57BL/6 embryos with a fragment of human genomic DNA containing either the entire hSod1 gene or the entire catalase gene as described previously. ApoE$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, Maine); these mice were generated by backcrossing to C57BL/6 for over 10 generations. In the present study, we used the following breeding strategies to generate ApoE$^{-/-}$ mice with overexpression of Cu/Zn-SOD and/or catalase. First, we crossedbreed ApoE$^{-/-}$ mice with hSod1/Tg$^{+}$ or hCat$^{+}$ mice to obtain mice with heterozygous knockouts for ApoE gene and heterozygous overexpression of human Sod1 (hSod1/Tg$^{+/+}$/ApoE$^{-/-}$) or catalase transgene (hCat$^{+/+}$/ApoE$^{-/-}$). We then bred ApoE$^{-/-}$ mice with hSod1/Tg$^{+/+}$/ApoE$^{-/-}$ or hCat$^{+/+}$/ApoE$^{-/-}$ mice to obtain mice with homozygous knockout for ApoE gene and heterozygous overexpression of Sod1 (hSod1/Tg$^{+/+}$/ApoE$^{-/-}$) or catalase transgene (hCat$^{+/+}$/ApoE$^{-/-}$). Finally, we obtained four lines of mice: (1) hSod1/Tg$^{+/+}$/ApoE$^{-/-}$, (2) hCat$^{+/+}$/ApoE$^{-/-}$, (3) mice with homozygous knockout for ApoE gene and heterozygous overexpression of both Sod1 and catalase transgenes (hSod1 $^{-/-}$/Cat$^{+/+}$/ApoE$^{-/-}$), and (4) ApoE$^{-/-}$ mice. The genotype of these mice was determined using polymerase chain reactions (PCR) of DNA obtained from mouse tail-clips. Mice overexpressing human Cu/Zn-SOD and catalase were identified by a 450-bp and a 219-bp PCR product, respectively, amplified from human sod1 and catalase gene. ApoE$^{-/-}$ mice were identified by PCR as described by Piedrahita et al.

**Measurement of Antioxidant Enzyme Activities**

The activities of Cu/Zn-SOD, Mn-SOD, extracellular SOD (EC-SOD), glutathione peroxidase-1 (GPx-1), and catalase were measured as described previously. Briefly, mouse aortas extracts containing 15 μg of protein were separated on native polyacrylamide gels. For detection of SOD activities, the gel was soaked in a solution containing nitroblue tetrazolium, riboflavin, and N,N,N',N'-tetramethyl-ethylenediamine (TMEDA). The gel was then illuminated at 560 nm in a light box for 15 minutes. Under these conditions, the area where the SOD activity was located showed a red color with the same staining solution used for the standard calibration curve produced from the intensities of purified Cu/Zn-SOD (Sigma Chemical Co) in the same gel and expressed as units/mg protein. For detection of GPx activity, the gel was incubated in a solution containing 0.008% cumene hydroperoxide and 1.5 mmol/L reduced glutathione for 10 minutes and then stained with a solution containing 1% ferric chloride and 1% potassium ferricyanide until the gel became dark green with yellow activity bands. For detection of catalase activity, the gel was soaked in a solution containing 0.003% hydrogen peroxide for 10 minutes and then stained with the same staining solution used for the GPx activity gel. The gel images were recorded and analyzed using the image acquisition system and software as described. The activity of GPx i.e., the catalytic activity of GPx1, was calculated based on the standard calibration curve, respectively produced from the intensities of purified GPx1 and catalase (Sigma Chemical Co) in the activity gels.

**Quantification of Atherosclerotic Lesions in the Mouse Aorta**

The aorta was cut at 2 mm from the heart. The proximal aorta attached with the heart was used to prepare cross-sections as described previously. Briefly, the heart was transversely sectioned immediately below and parallel with a plane formed by drawing a line between the atrial leaflets. The portion of the heart with the attached aorta was placed on a metal stub using OCT such that sectioning occurs from the attached aorta toward the root of the aorta where the aorta valves were attached. Sections (8 μm) were cut from the site where the aorta valve cups appear to the aorta root. Every other section was collected onto a set of microscope slides and stained with Oil-Red-O. The slides were viewed using a microscope (E600, Nikon Instruments Inc) equipped with a color digital camera (CoolSnaps, Nikon Instruments Inc) and a computer image acquisition system (MetaMorph image system, Nikon Instruments Inc). The average area (μm²) and morphological features (foam cell deposition, cholesterol clefts, acellular areas, and fibrous-caps) of the lesions in 16 sections were determined for each mouse. All measurements were conducted double blind, i.e., the person making the measurements was not aware of the source of the tissues and animals associated with each tissue were only identified when all tissues had been analyzed.
The distal aorta (2 mm from the heart to the bifurcation) was opened longitudinally using microscissors and pinned flat on a black wax surface in a dissecting pan under a dissecting microscope (SMZ1000, Nikon Instruments Inc). The en face preparation was fixed overnight and stained with Sudan IV. The photo image of the aorta was captured with a CoolSnaps digital camera mounted on the SMZ1000 dissecting microscope. The atherosclerotic lesion area and the total area of the aorta were measured using a MetaMorph image system. Data were expressed as the percentage of surface area of the aorta covered by atherosclerotic lesions. This measurement was also conducted double blind.

### Measurement of F2-Isoprostanes in the Mouse Aorta and Plasma
F2-isoprostanes are prostaglandin-like compounds that are produced nonenzymatically by free radical–catalyzed peroxidation of arachidonoyl lipids and believed to be one of the good indicators of lipid peroxidation in vivo. The levels of F2-isoprostanes in the mouse aorta and plasma were measured using a stable dilution isotope GC/MS assay. Aortas obtained from mice were minced in 2 mL of ice-cold HPLC-grade water containing 100 μM/L butylhydroxytoluene and 1 mmol/L ethylene diamine-tetraacetic acid (EDTA) and homogenized at 500 rpm for 5 minutes at ice temperature. After a known amount of [H3]F2-isoprostane internal standard was added to the aortic homogenate and plasma, total lipids were extracted and subjected to purification using TLC. F2-isoprostanes were then analyzed with GC/MS as described by Morrow and Roberts.

### Plasma Lipid Analysis
The levels of plasma cholesterol and triglycerides were measured by spectrophotometric quantification using reagents obtained from Sigma Chemical Co. For measuring cholesterol, the mixture of plasma and cholesterol-reaction reagent was incubated at 37°C for 30 minutes, and the absorbance was read at 530 nm with a Dynex microplate reader (Thermo Labsystems). For measuring triglycerides, the mixture of plasma and triglyceride-reaction reagent was incubated at 37°C for 10 minutes, and the absorbance was read at 530 nm. Plasma concentrations of cholesterol and triglycerides were determined based on the absorbance obtained by incubation of the cholesterol and triglyceride standards provided by Sigma. For analysis of the cholesterol distributed in various lipoproteins, a 100-μL plasma obtained from individual mouse was injected onto a Superose-6 column and fractionated with a fast performance liquid chromatography (Δkta FPLC 900, Amersham Pharmacia Biotech). Forty 0.5-mL fractions were collected, and tubes 11 to 40 were analyzed for cholesterol. Cholesterol content in various lipoproteins was calculated with the fraction as described by Hasty et al. Fractions 14 to 17 contained very low-density lipoprotein (VLDL) and chylomicrons, fractions 18 to 25 contained intermediate-density lipoprotein (IDL)/low-density lipoprotein (LDL), and fractions 26 to 40 contained high-density lipoprotein (HDL).

### Statistical Analysis
The data were reported as mean±SEM. The differences among hSod1Tg+/−/ApoE−/−, hCatTg+/−/ApoE−/−, hSod1+CatTgApoE−/−, and ApoE−/− mice were analyzed by multiple-factor analysis of variance followed by Shapiro-Wilk test. Differences were considered significant at a value of P<0.05.

### Results
Our previous study showed that the aortas of hSod1Tg+/− mice had 2.5-fold increase in the activities of Cu/Zn-SOD and catalase, respectively, but did not have significant alteration in the activities of other major antioxidant enzymes such as Mn-SOD, EC-SOD, catalase, and GPx1 when compared with their wild-type littermates. To establish if crossbreeding these transgenic mice into hSod1Tg+/− mice induces a compensatory alteration in the activities of antioxidant enzymes, we measured the activities of Cu/Zn-SOD, Mn-SOD, EC-SOD, catalase, and GPx1 in the aorta of hSod1Tg+/−/ApoE−/−, hCatTg+/−/ApoE−/−, hSod1+CatTg/ApoE−/−, and ApoE−/− mice. As data in Table 1 illustrate, aortas obtained from hSod1Tg+/−/ApoE−/−, hCatTg+/−/ApoE−/−, and hSod1+CatTg/ApoE−/− mice showed similar changes in the activities of the antioxidant enzymes as observed in the aorta of their hSod1Tg+/− and hCatTg+/− ancestors. For example, the activities of Cu/Zn-SOD and catalase in the aorta of hSod1Tg+/−/ApoE−/− mice were approximately 2.1-fold higher than those in the aorta of hSod1Tg+/− and hCatTg+/− mice, respectively, as compared with ApoE−/− control mice. The activities of Cu/Zn-SOD and catalase in the aorta of hSod1+CatTg/ApoE−/− mice were approximately 2.1-fold higher than those in the aorta of hSod1Tg+/− and hCatTg+/− mice, respectively, as compared with ApoE−/− control mice. In contrast, the activities of other antioxidant enzymes studied were not significantly altered in the aorta of hSod1Tg+/−/ApoE−/−, hCatTg+/−/ApoE−/−, and hSod1+CatTg/ApoE−/− mice when compared with their ApoE−/− littermates (Table 1). These observations suggest that overexpression of Cu/Zn-SOD and/or catalase does not result in a compensational regulation in other major antioxidant enzymes in the aorta of mice lacking ApoE.

This report examined the effect of overexpressing of Cu/Zn-SOD and/or catalase on atherosclerosis in ApoE−/− mice. Figure 1 shows examples of cross-sections and en face preparations of aortas obtained from hSod1Tg+/−/ApoE−/−, hCatTg+/−/ApoE−/−, hSod1+CatTg/ApoE−/−, and ApoE−/− mice. As data in Table 2 show, the severity of aortic atherosclerosis in hSod1Tg+/−/ApoE−/− mice was comparable to ApoE−/− mice. However, atherosclerotic lesions in hCatTg+/−/ApoE−/− and hSod1+CatTg/ApoE−/− mice differed from their ApoE−/− littermates. For instance, all the ApoE−/− mice developed both early stages of atherosclerotic lesions (eg, foam cells and free lipids) and advanced lesions (eg, fibrous caps and acellular areas). In contrast, only about half

### Table 1. Activities of Antioxidant Enzymes in the Mouse Aorta

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cu/Zn-SOD</th>
<th>Mn-SOD</th>
<th>EC-SOD</th>
<th>GPx-1</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>93.3±8.7</td>
<td>31.6±2.5</td>
<td>10.2±1.6</td>
<td>34.3±3.6</td>
<td>27.3±3.2</td>
</tr>
<tr>
<td>hSod1Tg+/−/ApoE−/−</td>
<td>215.6±17.4</td>
<td>33.8±5.3</td>
<td>9.7±2.0</td>
<td>32.6±4.0</td>
<td>26.2±3.5</td>
</tr>
<tr>
<td>hCatTg+/−/ApoE−/−</td>
<td>91.4±11.3</td>
<td>28.5±4.5</td>
<td>9.9±1.4</td>
<td>33.3±4.3</td>
<td>60.9±3.2*</td>
</tr>
<tr>
<td>hSod1+CatTg/ApoE−/−</td>
<td>193.6±16.8*</td>
<td>32.2±3.4</td>
<td>10.7±2.7</td>
<td>36.0±4.7</td>
<td>58.4±4.8*</td>
</tr>
</tbody>
</table>

Activities of Cu/Zn-SOD, Mn-SOD, EC-SOD, GPx-1, and catalase in the mouse aorta were determined using activity gels as described in the Materials and Methods and expressed as units/mg protein. Values are mean±SEM of five mice. *Statistically significant difference from wild-type mice (P<0.05).
of the hCatTg0+/Apoe−/− and hSod1+Catg/ApoE−/− mice showed fibrous caps and acellular areas in the atherosclerotic lesions located in the aortic sinus (Table 2). In addition, the surface area affected by atherosclerotic lesions in the entire aorta tree and the size of atherosclerotic lesions in the aortic sinus were significantly smaller in hCatTg0+/Apoe−/− and hSod1+Catg/ApoE−/− mice than in Apoe−/− control mice.

An elevated level of F2-isoprostanes has been found in the arterial wall has been suggested to play an important role in atherogenesis. To determine the effect of overexpressing Cu/Zn-SOD and/or catalase on lipid peroxidation, this report measured F2-isoprostanes in the plasma and aorta of Apoe−/− mice with or without overexpression of Cu/Zn-SOD and/or catalase. As data in the top panel of Figure 2 show, the level of F2-isoprostanes in the aorta and plasma obtained from the hCatTg0+/Apoe−/− and hSod1+Catg/ApoE−/− mice was significantly lower than that of Apoe−/− controls. Moreover, the plasma and aorta levels of F2-isoprostanes were significantly lower in hSod1+Catg/ApoE−/− mice than in hCatTg0+/Apoe−/− mice (Figure 2). The F2-isoprostane level in hSod1Tg0+/Apoe−/− mice was slightly less than that in Apoe−/− mice; however, the difference was not statistically significant (Figure 2). The aortic level of F2-isoprostanes showed significant correlation with the size of atherosclerosis in the mouse sinus (Figure 2, bottom panel). The plasma level of F2-isoprostanes also showed significant correlation with the size of atherosclerosis in the mouse sinus ($r^2$=0.639, $P<0.005$, data not shown).

An increase in plasma cholesterol and triglycerides has been suggested to be a risk factor for atherosclerosis. To determine whether the reduction of atherosclerotic lesions in hCatTg0+/Apoe−/− and hSod1+Catg/ApoE−/− mice was attributable to a change in plasma cholesterol and triglycerides, we measured the levels of plasma cholesterol and triglycerides in Apoe−/− mice with or without overexpression of Cu/Zn-SOD and/or catalase. As data in Table 3 show, the levels of cholesterol and triglycerides in hSod1Tg0+/Apoe−/−, hCatgTg0+/Apoe−/−, hSod1+Catg/ApoE−/−, and Apoe−/− mice were comparable. These results suggest that the reduced atherosclerotic lesions in hCatTg0+/Apoe−/− and hSod1+Catg/ApoE−/− mice are not due to alter the levels of plasma lipids.

**Discussion**

In the present study, we examined the effect of overexpressing Cu/Zn-SOD and/or catalase on atherosclerosis in...
Apoe<sup>−/−</sup> mice. As compared with Apoe<sup>−/−</sup> control mice, the Apoe<sup>−/−</sup> mice overexpressing catalase or doubly overexpressing Cu/Zn-SOD and catalase had smaller and relatively early stages of atherosclerotic lesions in their aorta, whereas the lesions in Apoe<sup>−/−</sup> mice overexpressing Cu/Zn-SOD alone were not significantly reduced. These observations suggest that H<sub>2</sub>O<sub>2</sub>, but not O<sup>2</sup>−, promotes the development of atherosclerosis in Apoe<sup>−/−</sup> mice. Currently, there have been two published studies in which transgenic mice have been used to study the effect of overexpression of Cu/Zn-SOD on atherosclerosis. In these studies, Trible et al<sup>11</sup> fed mice overexpressing Cu/Zn-SOD and their wild-type littermates a high fat diet and measured atherosclerosis in the proximal aorta. They observed that overexpression of Cu/Zn-SOD did not reduce the formation of fatty streaks induced by the high fat diet.<sup>12</sup> However, in a later study, Trible et al<sup>11</sup> observed that the extent of atherosclerotic lesions in the proximal aorta of the transgenic mice was significantly smaller than that in the aorta of wild-type mice when these mice were exposed to a single dose of x-ray and then placed on a high fat diet. These observations suggest that the atherogenic role of O<sup>2</sup>− varies in animal models, i.e., O<sup>2</sup>−-dependent processes contribute to the development of atherosclerosis in X-ray-exposed mice, but not in nonirradiated models such as fat-fed mice and Apoe<sup>−/−</sup> mice as shown in the present study.

Induction of lipid peroxidation has been proposed to be one of the mechanisms through which ROS potentiate the development of atherosclerosis. Consistent with this hypothesis, this report showed that the retarded development of atherosclerosis in the Apoe<sup>−/−</sup> mice overexpressing catalase or doubly overexpressing Cu/Zn-SOD and catalase was concomitant with a reduced F<sub>2</sub>-isoprostanes in the arterial wall. In a previous study, we observed that overexpression of Cu/Zn-SOD or catalase reduced vascular cell-induced LDL peroxidation.<sup>17</sup> The inhibitory effect of catalase was more than 2-fold higher than that of Cu/Zn-SOD.<sup>17</sup> These observations suggest that ROS, especially H<sub>2</sub>O<sub>2</sub>, play a role in vascular cell–mediated lipid oxidation. It has been suggested that the arterial pool of oxidized lipids is, at least in part, derived from lipids/lipoproteins that enter the intima from plasma as native forms but are then oxidized locally by cells in the arterial wall.<sup>2</sup> Thus, decrease in H<sub>2</sub>O<sub>2</sub> in the arterial wall could be an underlying cause for the reduced level of aortic F<sub>2</sub>-isoprostanes in the Apoe<sup>−/−</sup> mice overexpressing catalase or doubly overexpressing Cu/Zn-SOD and catalase. In the present study, we also observed a reduced level of plasma F<sub>2</sub>-isoprostanes in the Apoe<sup>−/−</sup> mice overexpressing catalase or doubly overexpressing Cu/Zn-SOD and catalase when compared with Apoe<sup>−/−</sup> controls. The plasma and aortic levels of F<sub>2</sub>-isoprostanes were significantly correlated (data not shown). The plasma-born oxidized lipids have been suggested to be a source of the arterial pool of oxidized lipids.<sup>19,33</sup> Thus, the reduction in plasma F<sub>2</sub>-isoprostanes might be also responsible for the reduced level of aortic F<sub>2</sub>-isoprostanes in Apoe<sup>−/−</sup> mice overexpressing catalase or both Cu/Zn-SOD and catalase.

Oxidized lipids and/or oxidized lipoproteins accumulated in the arterial wall have been suggested to give rise to atherosclerosis by inducing a series of cellular responses such as injuring ECs, recruiting inflammatory cells into the intima, and inducing vascular SMC proliferation (see
reviews. \cite{1,2}). Accumulating evidence suggests that the atherogenic action of oxidized lipids is, at least in part, associated with its ability to induce ROS in vascular cells. \cite{34,35} The increased ROS in vascular cells are believed to function as second messengers of the oxidized lipids and induce expression of a variety of proteins that are thought to be involved in recruitment of inflammatory cells to the vessel wall, proliferation, and death of vascular cells. \cite{34,36} Data from our previous studies have shown that overexpression of catalase suppresses oxLDL-induced aortic SMC death. \cite{17} Overexpression of catalase also has been shown to inhibit oxLDL-induced leukocyte adhesion to ECs and epidermal growth factor-induced SMC proliferation. \cite{38} These observations strongly support the notion that H$_2$O$_2$ plays a role in the pathogenesis of atherosclerosis. Therefore, reduction in H$_2$O$_2$-involved cellular responses could be responsible for the retarded development of atherosclerosis in ApoE$^{-/-}$ mice overexpressing catalase or both Cu/Zn-SOD and catalase.

In summary, this report provided the first evidence that overexpression of catalase retarded the development of atherosclerosis and reduced the level of F$_2$-isoprostanes in the aorta of the ApoE$^{-/-}$ mice. These data, together with our previous observations that overexpression of catalase reduced the responses of vascular cells to oxidized lipids, \cite{17,37,38} suggest that H$_2$O$_2$ are involved in the pathogenesis of atherosclerosis by inducing lipid peroxidation and increasing the sensitivity of vascular cells to oxidized lipids. These data suggest that endogenously produced H$_2$O$_2$ is, at least in part, responsible for the formation of oxidized lipids and the development of atherosclerosis in ApoE$^{-/-}$ mice. This report also showed that overexpression of Cu/Zn-SOD alone did not reduce the level of F$_2$-isoprostanes and atherosclerosis in ApoE$^{-/-}$ mice, suggesting that O$_2^-$-dependent processes might not predominate in promoting lipid peroxidation and atherogenesis in these mice.

Acknowledgments

This study was supported by NIH grants HL071525 and GM08037 (to Z.M.G.) and HL076623 (to H.Y.).

References

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Circ Res. published online November 4, 2004;

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