Effect of Doxazosin on Endothelial Dysfunction in Hypercholesterolemic/Antioxidant-Deficient Rats
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Hypertension, hypercholesterolemia, atherosclerosis, and coronary heart disease are associated with abnormal endothelium-dependent, nitric oxide-mediated vasorelaxation. In rats, hypercholesterolemia in combination with deficiencies of vitamin E and selenium results in increased endogenous lipid oxidation and endothelial dysfunction. Two hydroxymetabolites of doxazosin, an $\alpha_1$-adrenergic blocking antihypertensive agent, inhibit human lipid oxidation in vitro in a dose-dependent fashion. The present studies were performed to determine the effect of in vivo treatment with doxazosin on endothelial dysfunction in hypercholesterolemic/antioxidant-deficient rats. Dahl rats were fed 1) a standard diet, 2) a high cholesterol (4%) diet, or 3) a high cholesterol, vitamin E- and selenium-deficient diet. A subgroup of animals in each group were administered doxazosin (3.5 mg/100 g/day) for 16 weeks. In the aortas, vascular relaxations induced by acetylcholine were significantly decreased ($P < .05$) in high cholesterol/antioxidant-deficient rats compared with normal and high cholesterol animals. Doxazosin treatment prevented the impairment in endothelium-dependent vascular relaxation in the high cholesterol/antioxidant-deficient group. Vasorelaxation in response to the exogenous nitric oxide donor diethylamine ninoate, which was significantly impaired ($P < .05$) in aortas from high cholesterol/antioxidant-deficient animals compared with normal and high cholesterol animals, was normalized in aortas from high cholesterol/antioxidant-deficient animals that had received doxazosin. The antioxidant effect of doxazosin may have therapeutic implications in diseases associated with endothelial dysfunction linked to products of lipid oxidation. Am J Hypertens 1997; 10:1257–1262 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Endothelium, nitric oxide, doxazosin, vasorelaxation, hypercholesterolemia, antioxidants.
smooth muscle. In subsequent studies by numerous research groups, the endothelium was shown to continuously release a labile vasorelaxant, which was called initially endothelium-derived relaxing factor (EDRF). It is now known that EDRF is nitric oxide (NO) or an NO-containing compound, such as an S-nitrosothiol. EDRF/NO also inhibits platelet aggregation and adhesion to the endothelium. NO is produced from the oxidation of the guanidino nitrogen of l-arginine by the enzyme NO synthase. NO synthase is stimulated by a number of factors, including acetylcholine, bradykinin, serotonin, histamine, thrombin, aggregating platelets, and shear stress resulting from increased blood flow. NO activates the soluble guanylate cyclase of vascular smooth muscle, resulting in an increase in cyclic guanosine monophosphate (cGMP) formation from guanosine triphosphate, a decrease in the intracellular calcium ion concentration, and vascular relaxation. The endothelium also synthesizes other vasoactive and antiplatelet substances, such as prostacyclin, endothelium-derived hyperpolarizing factor, endothelin, and vasoconstricting prostanoids, which act with EDRF/NO to regulate vascular tone and platelet activity. Endothelial dysfunction has been studied extensively in animal models of hypercholesterolemia and atherosclerosis and in arteries from patients with these diseases. Evidence suggests that oxidized low density lipoprotein (LDL) cholesterol attenuates EDRF/NO-induced activation of cGMP. The results of a study by Chin and associates support a mechanism wherein the lipid component of oxidized LDL inactivates EDRF/NO after its release from endothelial cells. It has been suggested that lysophosphatidylcholine (lysolecithin), which forms on oxidation of lecithin in LDL and stimulates superoxide anion formation, may be involved in this process. Superoxide anion reacts with and inactivates EDRF/NO. Low molecular weight antioxidants and probucol, a cholesterol-lowering drug with antioxidant properties, have been found to protect LDL against oxidation in several animal models. Furthermore, the 6’- and 7’-hydroxymetabolites of doxazosin, an α1-adrenergic blocking antihypertensive agent, have been shown to inhibit human lipid oxidation in vitro in a dose-dependent fashion. These two metabolites of doxazosin are formed by hydroxylation of the benzodioxan moiety, which is unique to doxazosin.

Unlike other species, rats are resistant to the development of vascular dysfunction and atherosclerosis induced by hypercholesterolemia. In a previous study in hypercholesterolemic rats, coexisting deficiencies of vitamin E and selenium were required to produce impaired endothelium-dependent vasorelaxation mediated by EDRF/NO. Endogenous lipid oxidation was increased in these hypercholesterolemic/antioxidant-deficient animals, suggesting that antioxidant protection is important in maintaining normal vascular tone. In the present studies, this in vivo model of vascular disease was used to evaluate the effect of doxazosin on endothelial dysfunction.

METHODS

Animals Male, 6-week-old, 190 to 210 g, Dahl salt-sensitive rats from the Brookhaven strain were purchased from Harlan Sprague Dawley (Indianapolis, IN). Rats were housed five animals to a cage and had free access to water. The animal facilities were accredited by the American Association for Accreditation of Laboratory Animal Care, and the animal studies were approved by the Institutional Animal Care and Use Committee.

Six groups were studied: 1) a normal cholesterol (control) group (n = 6) fed standard rat chow containing 0.5% NaCl, 0.3 mg/kg selenium, and 50 mg/kg vitamin E (certified rodent chow No. 5002, Purina Mills, St. Louis, MO); 2) a normal cholesterol group (n = 10) that received doxazosin, 3.5 mg/100 g daily, by gavage; 3) a high cholesterol group (n = 6) fed a diet containing 4% cholesterol and 1% cholic acid, with a selenium content of 0.3 mg/kg and a vitamin E content of 50 mg/kg (diet No. 91026, Teklad Premier Laboratory Diets, Madison, WI); 4) a high cholesterol group (n = 10) that received doxazosin, 3.5 mg/100 g daily, by gavage; 5) a high cholesterol and selenium/vitamin E-deficient group (n = 6) fed a high cholesterol diet, as described above, that contained no selenium or vitamin E (diet No. 91027, Teklad Premier Laboratory Diets); and 6) a high cholesterol and selenium/vitamin E-deficient group (n = 10) that received doxazosin, 3.5 mg/100 g daily, by gavage. Both experimental diets, which had sodium, mineral, and protein contents that were similar to the standard diet, were given for 16 weeks.

Systolic blood pressure was measured in unanesthetized rats by the tail-cuff method using a Physiograph MK IV (Narco Biosystems, Houston, TX). After an overnight fast, rats were anesthetized with sodium pentobarbital, 50 mg/kg intraperitoneally. After making a midline incision, blood was collected from the abdominal aorta for biochemical analyses. The rats were killed by exsanguination, and thoracic aortas were excised and processed for organ chamber studies.

Biochemical Analysis Total plasma cholesterol was analyzed with an Ektachem 700XR (Eastman Kodak Co., Rochester, NY). Serum vitamin E levels were measured using high performance liquid chromatography.

Organ Chamber Experiments Aortic rings were suspended between two stirrups in organ chambers filled with oxygenated modified Krebs-Ringer bicarbonate.
Relaxations to sodium nitroprusside (10⁻⁴ mol/L) were determined in rings precontracted to 60% to 70% of the maximal norepinephrine-induced contraction. All data are expressed as mean ± SEM. The statistical significance of differences between groups was assessed by analysis of variance. The criterion for significance was *P < .05*, †*P < .05* v NChol and HChol-Def, ‡*P < .05* v NChol and HChol-Def, ††*P < .05* v HChol and HChol-Def, and †††*P < .05* v HChol and HChol-Def.

### RESULTS

Mean body weights, systolic blood pressure measurements, plasma total cholesterol levels, and serum vitamin E levels in the six animal groups are shown in Table 1. Similar levels of total cholesterol were found in the high cholesterol and the high cholesterol/antioxidant-deficient animals. The high levels of vitamin E in the high cholesterol animals are attributable to lipoprotein binding of vitamin E, which results in elevated absolute values in hypercholesterolemia. All animal groups remained normotensive and had similar mean systolic blood pressure measurements.

Acetylcholine (10⁻⁹ to 10⁻⁴ mol/L) produced relaxation of precontracted thoracic aorta rings with intact endothelium from all groups of rats (Figure 1). However, acetylcholine-induced relaxations were significantly decreased in rings from high cholesterol/antioxidant-deficient animals (*P < .05*) compared with those from normal cholesterol and high cholesterol animals.

### Statistical Analysis

All data are expressed as mean ± SEM. The statistical significance of differences between groups was assessed by analysis of variance. The criterion for significance was *P < .05*.
azosin. Vascular relaxation induced by DEANO was significantly decreased \((P < .05)\) in aortas from high cholesterol/antioxidant-deficient animals compared with normal cholesterol animals (Figure 3). However, DEANO-induced vascular relaxation was normalized in aortas from high cholesterol/antioxidant-deficient animals that had been treated with doxazosin \((P < .05)\) versus high cholesterol/antioxidant-deficient.

**DISCUSSION**

The oxidation of LDL cholesterol by the vascular endothelium is thought to play a role in the pathogenesis of endothelial dysfunction that is associated with hypertension, hypercholesterolemia, atherosclerosis, and coronary heart disease.\(^{12,13,28}\) In the present studies, hypercholesterolemic/antioxidant-deficient rats demonstrated impaired vasorelaxation of thoracic aortas in response to acetylcholine but not in response to sodium nitroprusside, suggesting a dysfunction of endothelium-dependent vascular relaxation without a dysfunction of vascular smooth muscle. These results confirm those of a previous study,\(^{23}\) which also demonstrated that lipid oxidation was increased in these hypercholesterolemic/antioxidant-deficient rats and
that cyclooxygenase products and endothelin-1 did not play a role in the observed endothelial dysfunction. Subsequent studies have shown that impaired nitric oxide synthase activity is not responsible for the endothelial dysfunction in these animals (unpublished data).

The present studies demonstrated impaired vasorelaxation in response to the exogenous nitric oxide donor DEANO in hypercholesterolemic/antioxidant-deficient rats. The differences observed between the responses to sodium nitroprusside and DEANO can be explained by the different mechanisms by which nitric oxide is released from these two compounds. Sodium nitroprusside releases nitric oxide within the vascular smooth muscle. Nitric oxide is released from DEANO in the organ bath; the nitric oxide then diffuses through the vascular endothelium and smooth muscle. The results of the present study suggest that nitric oxide released from DEANO is inactivated, possibly by oxidized lipoproteins or superoxide anion, as it diffuses through the vascular wall.

Doxazosin treatment did not increase the sensitivity or maximal relaxation to acetylcholine in normal cholesterol animals. On the other hand, doxazosin treatment prevented the abnormal endothelium-dependent vasorelaxation in response to acetylcholine and DEANO in hypercholesterolemic/antioxidant-deficient rats. The 6’- and 7’-hydroxymetabolites of doxazosin have been shown to inhibit the in vitro oxidation of human LDL.20 Doxazosin may prevent the development of endothelial dysfunction in the present rat model by the inhibitory action of its metabolites on lipoprotein oxidation. The hydroxylation of doxazosin occurs in its benzodioxan moiety, which is not a part of the chemical structure of other α1-adrenergic blockers, suggesting that this antioxidant effect may be unique to doxazosin. Because LDL oxidation is generally accepted to be a critical step in the atherogenic process, therapeutic agents with the ability to protect lipoproteins from oxidative metabolism may provide a means to limit the development or progression of atherosclerosis. Thus, the antioxidant effect of doxazosin metabolites may have future implications in the therapeutic management of atherosclerosis as well as other diseases characterized by an impairment of nitric oxide-mediated vasorelaxation.

REFERENCES