Effects of Hypertension on Aortic Antioxidant Status in Human Abdominal Aneurysmal and Occlusive Disease (43188)

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Abstract. **The biochemical mechanisms by which hypertension accelerates atherosclerosis and increases the risk of aortic aneurysm rupture are poorly understood. This study evaluates the effects of hypertension on aortic trace element concentrations and antioxidant status in tissue removed from 26 normotensive (NT) and 20 hypertensive (HT) patients. Twenty-seven of 46 patients (59%) had aneurysmal (AA), and 19 of 46 (41%) had occlusive disease (OD).**

Aortic iron concentrations were markedly higher in both OD and AA tissue compared with controls. A similar trend was observed with copper concentrations, with the highest elevations observed in HT AA tissues. No significant differences were observed in zinc concentrations, except that HT AA aorta had significantly lower zinc levels than either OD or control tissue. Aortic ascorbic acid concentrations in diseased aorta were lower than those of controls, but independent of blood pressure. Copper-zinc-superoxide dismutase activity was similarly reduced, with the lowest activity observed in diseased aorta from HT patients. Only HT AA aorta had significantly higher manganese-superoxide dismutase activity than controls.

The aortas of patients with AA had significantly lower amounts of elastin and greater elastase activity than either controls or those with OD. However, the differences were independent of blood pressure. Hypertensive patients with OD and AA had 31% more and 27% less aortic collagen, respectively, than their NT counterparts ($P < 0.05$). These data suggest that the reduction in aortic collagen and elastin in HT patients with AA compared with their NT counterparts may explain the larger size of aneurysms and predispose to their eventual rupture. Furthermore, the diminished antioxidant status associated with HT predisposes to lipid peroxidation, which contributes to the acceleration of these processes.

Our studies were conducted in patients with established aortic aneurysmal and occlusive disease. Whether these observations are pertinent to the pathogenesis of AA and OD remains unclear and merits further study. [P.S.E.B.M. 1991, Vol 196]

Hypertension, a known risk factor in the progression of atherosclerosis, is associated with a 5-fold increase in the risk of stroke, a 2- to 3-fold increase in the incidence of ischemic heart disease, and a 2-fold increase in peripheral vascular disease when compared with normotensive individuals (1–3). In addition, hypertension is also a significant risk factor in the growth and/or rupture of abdominal aortic aneurysms (AA) and is present in 50% of patients with AA (4, 5). These increases in the clinical complications induced by hypertension have been ascribed to acceleration of the atherosclerotic process (6, 7). Although AA and occlusive disease (OD) of the infrarenal aorta share many of the manifestations of atherosclerosis, there is increasing evidence indicating biochemical and structural differences between these two varieties of aortic disease (8).

Morphologically, AA is characterized by attenuation of the aortic wall due to a reduction in smooth muscle and elastin content. By contrast, in OD there is an increase in the thickness of the aortic wall with a

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well-preserved media containing smooth muscle cells. These differences in the presentation of aortic atherosclerosis may be explained by alterations in the balance between collagen and elastin synthesis and degradation as proposed by Busuttil and Cardenas (8). Aortic lesions in animals fed an atherogenic diet are characterized by increased aortic collagen and elastin synthesis, lipid deposition, and resemble those seen in patients with OD (9–12). Similar alterations in aortic collagen and elastin metabolism have been observed in hypertensive animals (13–15).

Trace elements such as copper, zinc, and iron and ascorbic acid are important co-factors in the synthesis of collagen and elastin (16) and, along with superoxide dismutase, modulate oxygen-free radical reactions implicated in atherosclerosis (17). Furthermore, abnormal copper and zinc metabolism has been implicated in the pathogenesis of both abdominal aortic aneurysms and chronic hypertension (18–20).

The present study examines the effects of hypertension on trace element and ascorbic acid concentrations, collagen and elastin content, and elastase and superoxide dismutase activity in aortic tissue removed at surgery from normotensive and hypertensive patients with AA and OD as an initial step in the investigation of the possible mechanisms contributing to these diseases.

Materials and Methods

Clinical Data. The clinical risk factors of atherosclerosis in 46 male veteran patients from whom operative specimens of aortic tissue were obtained are presented in Table I. Aneurysm size was determined by abdominal ultrasound or computerized tomography. Hypertension was defined by a systolic blood pressure greater than 140 mm Hg and a diastolic blood pressure of greater than 90 mm Hg or by the need for chronic antihypertensive therapy. Routine electrocardiogram, chest x-ray, serum chemistries, and hematologic tests were performed on all patients. Aortograms, including runoffs, were performed by the Seldinger technique in all patients with aneurysmal and occlusive disease.

Aortic Tissue Specimen Acquisition. *Autopsy tissue.* A ring of aorta was removed from nine young male accident victims aged 19–40 years within 24 hr of death. Specimens were excised 2 cm below the renal arteries and frozen at −70°C until assayed. Although no data on atherosclerosis risk factors were available from these patients, only those specimens with no gross signs of atherosclerosis were used. Information regarding these control specimens have been reported previously (18).

Operative specimens. Samples of aorta were obtained at the time of operation from 19 patients with occlusive disease and 27 patients with abdominal aortic aneurysms. Specimens were taken from the abdominal aorta at least 2 cm below the origin of the renal arteries and included the entire thickness of the aortic wall. A portion of the specimen was submitted for histologic examination. The remainder of the specimen was carefully rinsed of blood, and the surrounding fibrofatty tissue was removed. The specimens were then snap-frozen in liquid nitrogen and stored at −70° until assayed.

Biochemical Studies. *Trace metals.* Tissue concentrations of iron, copper, and zinc were determined by flame atomic absorption spectroscopy. The addition of known concentrations of selected metals as standards indicated that recoveries ranged from 98 to 102% of all elements (21).

Ascorbic acid. Tissue ascorbic acid levels were determined according to the method of Day *et al.* (22), as modified for tissue homogenates (23).

Superoxide dismutase. Superoxide dismutase (SOD) activity was determined in aortic tissue samples homogenized in 0.25 *M* sucrose to 10% (w/v) and sonicated three times at maximum frequency for 5 sec each. The homogenates were then centrifuged at 4°C for 30 min at 10,000*g*. The total SOD activity was determined by inhibition of the autoxidation of pyrogallol as described previously (24). Mn-SOD activity was measured under the same conditions except that 1 m*M* KCN was added to the assay buffer to inhibit Cu-Zn SOD activity. Cu-Zn activity was expressed as the difference between total SOD activity and Mn-SOD activity.

Lipid peroxidation. Lipid peroxide protein adducts

Table I. Atherosclerosis Risk Factors in Patients with Occlusive and Aneurysmal Disease

	Occlusive disease		Aneurysmal disease	
	NT (n = 11)	HT (n = 8)	NT (n = 15)	HT (n = 12)
Age (years)	64.5 ± 8.0[a]	63.4 ± 9.2	66.8 ± 8.6	65.8 ± 7.6
Smoking (pack/year)	67.6 ± 30 (10)	52.5 ± 24 (8)	52.2 ± 24 (14)	54.3 ± 18.3 (10)
Systolic BP (mm Hg)	132 ± 5	158 ± 8[b]	129 ± 5	153 ± 6[b]
Diastolic BP (mm Hg)	76 ± 3	93 ± 5[b]	74 ± 2	82 ± 4

[a] Data expressed as mean ± SD.
[b] $P < 0.05$.

were determined fluorometrically using the method described by Tappel (25) as described previously (26).

Collagen and elastin content and elastase activity. The content of mature elastin from the same specimens was determined according to the method of O'Dell *et al.* (27). Estimates of elastin content were derived from the measurement of ninhydrin-reactive nitrogen (28). The collagen content of the aortic specimens was expressed as the hydroxyproline content of the supernatant fractions collected during the isolation of elastin (29). Homogenized aortic tissue was repeatedly washed in saline to remove as much blood as possible. The clear supernatant was then assayed for hemoglobin and albumin, and no detectable levels of these blood proteins were found. The resulting pellet of tissue was further extracted with 0.5 *M* sodium acetate at pH 4.5 as described by Bellon *et al.* (30), and the supernatant fraction was then assayed for elastase activity according to the method of Bieth *et al.* (31) using succinyl $(ala)_3$-*p*-nitroanalide as substrate. Elastolytic activity was expressed as units/g wet weight of aorta.

Statistical Analysis. Data were analyzed by analysis of variance. Student-Newman-Keuls test was used to determine statistical significance within groups, with $P < 0.05$ considered significant.

Results

The clinical risk factors of the patients are listed in Table I. There were no significant differences in age or the number of cigarettes smoked by patients with OD and AA. Three patients, two with OD and one with AA, had insulin-dependent diabetes.

No differences in systolic or diastolic blood pressure in normotensive (NT) patients were observed. There was a significant difference in the systolic blood pressure in both OD and AA patients defined as hypertensive (HT) compared with those defined as NT ($P < 0.05$), whereas the diastolic blood pressure was only significantly higher in OD patients with HT ($P < 0.05$). The antihypertensive medications taken by the patients included the usual spectrum of diuretics, β-blockers, angiotensin-converting enzyme inhibitors, and vasodilators. Four of eight (50%) patients with OD and 4 of 12 (33%) with AA were taking diuretics.

The mean transverse aortic diameter was 6.5 ± 0.7 cm for NT and 8.3 ± 0.8 cm for HT patients with AA.

Trace Element Concentrations. The aortic trace element concentrations are represented in Figure 1. There were no significant differences in aortic copper concentrations between control and normotensive patients with OD or AA. However, AA and OD hypertensive patients had significant elevations in aortic copper when compared with controls ($P < 0.05$). Zinc concentrations were similar in all groups, except for a significant reduction in AA patients with HT ($P < 0.05$), when compared with controls. Normotensive patients

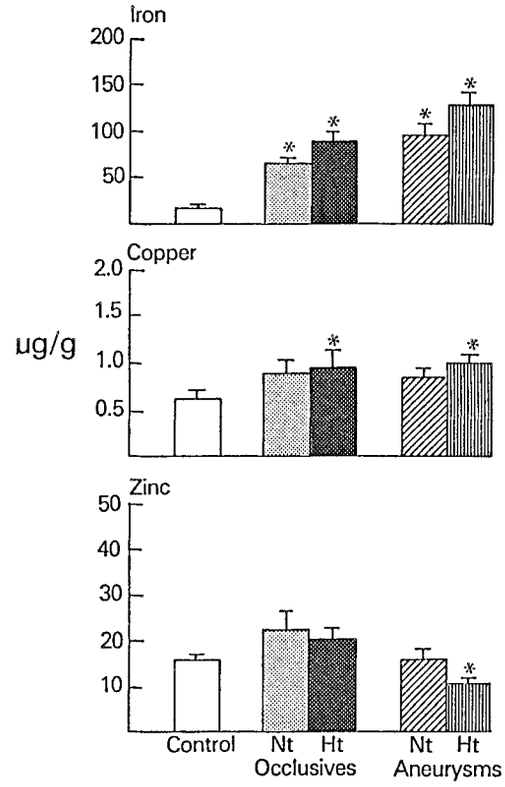

Figure 1. Comparison of aortic iron, copper, and zinc concentrations in normotensive and hypertensive patients with occlusive and aneurysmal disease. Data expressed as mean ± SE number of samples/group, as in Table II. Control data from Dubick *et al.* (18).

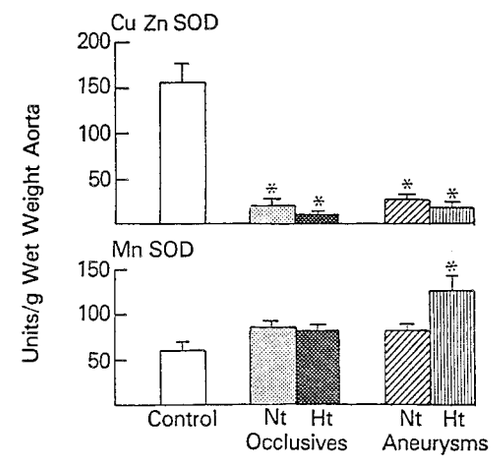

Figure 2. Superoxide dismutase activity and ascorbic acid concentrations in patients with occlusive and aneurysmal disease. Data expressed as mean ± SE. Control data from Dubick *et al.* (18).

with aneurysms had a 6-fold and HT 8-fold greater iron concentrations than controls ($P < 0.05$).

Superoxide Dismutase Activity and Ascorbic Acid Concentrations. The Cu-Zn SOD activity was significantly reduced in all diseased aortic tissue when compared with controls, irrespective of blood pressure (Fig. 2). In contrast, no significant differences in Mn-SOD activity were observed in any group, except Mn-

SOD activity was significantly elevated in AA patients with HT ($P < 0.05$). Ascorbic acid concentrations were significantly reduced in all diseased aortas, irrespective of blood pressure ($P < 0.05$).

Lipid Peroxidation. Preliminary assay of tissue lipid peroxides demonstrated a 6-fold increase in fluorochromes in the six atherosclerotic aortic tissue specimens examined (133 ± 28) compared with the three controls (23 ± 2) Fu/g. We are presently assaying additional samples to determine the effects of hypertension on this parameter. The preliminary data, however, support the observed decrease in antioxidant levels in diseased versus nonatherosclerosic control tissue.

Collagen and Elastin Content and Elastase Activity. The aortic collagen, elastin content, and elastase activity are presented in Table II. There were no significant differences in total collagen content between control and OD and AA aortic tissue. However, when compared with their corresponding NT group, hypertensive OD patients had 31% more collagen ($P < 0.05$), whereas AA patients with HT had 27% less aortic collagen ($P < 0.05$). In addition, there was a 42% reduction in aortic elastin content in OD and an 8-fold reduction in AA patients, when compared with controls ($P < 0.05$). Furthermore, AA patients had significantly less (4.5-fold) elastin than OD patients ($P < 0.05$). This reduction in elastin was independent of blood pressure in both groups.

By contrast, elastase activity was significantly increased in all diseased aortic tissue ($P < 0.05$). Aortic elastase activity in normo- and hypertensive OD patients was 5-fold and 7-fold higher, respectively, than in controls ($P < 0.05$). In AA patients, elastase activity was 11-fold and 12-fold higher in NT and HT patients, respectively, than in controls ($P < 0.05$). Elastase activity in both AA groups was approximately 2-fold higher ($P < 0.05$) than in the corresponding OD groups.

Discussion

Epidemiologic and clinical studies have demonstrated that hypertension accelerates the progression of atherosclerosis and increases the rate of growth and risk of rupture of abdominal aortic aneurysms (4, 5, 32). Although the mechanisms by which these effects are mediated are poorly understood, increased wall stress, endothelial cell damage, free radical reactions, and altered collagen and elastin synthesis and degradation have all been implicated as possible etiologic factors (8, 17, 33, 34).

The present study was undertaken to examine the metabolism of trace elements, antioxidants, and proteases in aortic tissue removed from patients with occlusive and aneurysmal disease that are known to be involved in connective tissue metabolism and are altered by atherosclerosis and hypertension.

Aortic elastin concentrations were significantly lower in AA patients than in both OD patients and controls, consistent with previous observations by us and others (35–37). Morphologically, as described previously (8), the elastin present is fragmented, patchy in distribution, and entirely absent in some areas. These alterations in aortic elastin observed in patients with aneurysms is associated with significantly higher elastase activity than that observed in both occlusive and control aortas and is consistent with data reported previously (8, 35–39). Yamada *et al.* (40, 41) and Ito *et al.* (42) have reported significantly higher aortic elastase activity in hypertensive animals when compared with normotensive controls. There were no significant differences in aortic elastase activity in AA and OD pa-

Table II. Aortic Elastin and Collagen Content and Elastase Activity

	n	Collagen (mg/g)	Elastin (mg/g)	Elastase activity (units/g)
Controls	9	66.1 ± 5.8[a]	27.3 ± 4.1	0.33 ± 0.05
Occlusive disease				
Normotensive	11	54.0 ± 4.9	16.0 ± 3.4[b]	1.73 ± 0.24[b]
Hypertensive	8	78.6 ± 2.0[c]	15.7 ± 2.6[b]	2.32 ± 0.23[b]
Aneurysmal disease				
Normotensive	15	68.6 ± 3.0	4.50 ± 0.45[b,d]	3.76 ± 0.58[b,d]
Hypertensive	12	50.0 ± 2.2[c]	3.55 ± 0.60[b,d]	4.14 ± 0.73[b,d]

[a] Data expressed as mean ± SE.
[b] $P < 0.05$ from controls.
[c] $P < 0.05$ from corresponding normotensive group.
[d] $P < 0.05$ from occlusive disease.

tients with HT when compared with their normotensive counterparts. Ito *et al.* (42) have reported reduced aortic elastase activity in spontaneously hypertensive rats receiving antihypertensive treatment. Whether antihypertensive therapy in AA and OD patients affected the levels of aortic elastase activity measured requires further investigation.

Sumner *et al.* (36) observed an increase in aortic collagen in occlusive disease of the aorta, whereas more recent studies of AA have reported conflicting results, demonstrating either an increase or a decrease in the collagen content of aneurysmal aortas. Aortic collagen content (per g wet weight) in both AA and OD patients was similar to that of controls. However, when compared with their NT counterparts the aortas of HT patients with OD contained 31% more and those with AA 27% less collagen. A possible reason for the differences in collagen content in OD and AA patients with HT may be the presence of collagenase activity in aneurysmal aortic tissue as reported by Busuttil *et al.* (43). The highest levels of collagenase activity were associated with ruptured aneurysms, whereas collagenase activity was absent in OD aortas (43, 44). Yamada *et al.* (41) have demonstrated higher aortic collagenase activity in spontaneously hypertensive rats when compared with controls. Whether similar increases in collagenase activity exist in human aortic tissue in patients with HT remains to be determined.

Dobrin (45), using collagenase- and elastase-digested dog carotid and human iliac arteries, has demonstrated that a decrease in elastin predisposes to dilation, whereas the amount of collagen present determines if the vessel ultimately ruptures. These data suggest that the reduction in aortic collagen and elastin may explain the larger size and increased risk of rupture of AA in patients with HT. The recent studies by Leach *et al.* (46), demonstrating the reduction in the growth rate of aneurysms in patients treated with β-blockers, is interesting in view of these findings and needs further evaluation.

It is known that oxidative mechanisms may augment the activity of proteolytic enzymes, perhaps through inhibition of specific inhibitors (47, 48). Therefore, to further examine possible mechanisms in addition to those associated with altered collagen and elastin metabolism in aortic disease, we determined the concentrations of co-factors involved both in free radical reactions and connective tissue metabolism.

Recent studies have suggested that aortic aneurysms may be associated with, or be a consequence of, copper deficiency (16, 49). Tilson (50) reported low concentrations of copper in the skin and liver of patients with abdominal aortic aneurysms and postulated that copper deficiency, known to be associated with aneurysm formation in blotchy mice, may be a predisposing factor in the development of abdominal aortic aneurysms in humans. In the present study, aortic copper was increased in all diseased aortic tissue when compared with controls and was significantly higher in hypertensive patients with OD and AA. These data agree with the observations of Senapti *et al.* (51). The increase in copper and the marked reduction in Cu-Zn-SOD activity in diseased aortas compared with controls suggests a possible functional copper deficiency. These alterations in Cu and Cu-Zn-SOD activity could affect connective tissue maturation, as well as contribute to oxidative mechanisms that may influence the disease process adversely. The compensatory increase in Mn-SOD activity in diseased aorta, and particularly in AA patients with HT, is consistent with generation of oxygen-free radicals, observed under conditions of oxidative stress (52).

Zinc concentrations were 38% lower in the aortic tissue of hypertensive patients with atherosclerosis. Low serum zinc levels have been observed in experimental animals and in patients with hypertension (19). Szebeni *et al.* (53) have recently demonstrated that zinc inhibits lipid peroxidation, suggesting that the low levels of zinc in AA patients with HT may also be indicative of or promote increased lipid peroxidation.

The elevation in aortic iron is of particular interest because the metal is a catalyst in the formation of hydroxyl radicals from hydrogen peroxide via the Haber-Weiss or Fenton reactions. The high concentrations of iron observed in diseased aortic tissue in this study cannot be attributed to hemoglobin inasmuch as the specimens were essentially free of this protein when assayed. Investigations are currently underway to determine whether abdominal iron metabolism in aortic tissue contributes to lipid peroxidation.

The reduction in ascorbic acid in atherosclerotic tissue may be indicative of an increase in the generation of free radicals or simply reflect reduced levels of the vitamin known to occur in cigarette smokers, since all but four of the patients in this study smoked (54, 55). The combination of high iron and reduced ascorbate concentrations provides additional indirect evidence for oxygen-free radical mechanisms and is supported by our preliminary observation of increased lipid peroxidation in diseased aortic tissue.

Although statistically significant differences could not be demonstrated between HT and NT patients with aortic disease in all of the parameters measured, the trends observed support our hypothesis that hypertension may influence AA and OD through its effects on oxidative mechanisms in addition to those on collagen and elastin.

Finally, we recognize that the changes observed in this study represent the end result of the atherosclerotic process, and their exact role in the pathogenesis of AA and OD remains undetermined. However, we believe that the definition of the underlying abnormalities will

lead to the development of experimental models to study the effects of hypertension on the progression of aortic atherosclerosis, as well as lead to the development of preventive modalities and/or biochemical markers.

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