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Long-Term Ascorbic Acid Administration Reverses Endothelial Vasomotor Dysfunction in Patients With Coronary Artery Disease

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Background—Loss of endothelium-derived nitric oxide (EDNO) contributes to the clinical expression of coronary artery disease (CAD). Increased oxidative stress has been linked to impaired endothelial vasomotor function in atherosclerosis, and recent studies demonstrated that short-term ascorbic acid treatment improves endothelial function.

Methods and Results—In a randomized, double-blind, placebo-controlled study, we examined the effects of single-dose (2 g PO) and long-term (500 mg/d) ascorbic acid treatment on EDNO-dependent flow-mediated dilation of the brachial artery in patients with angiographically established CAD. Flow-mediated dilation was examined by high-resolution vascular ultrasound at baseline, 2 hours after the single dose, and 30 days after long-term treatment in 46 patients with CAD. Flow-mediated dilation improved from $6.6\pm3.5\%$ to $10.1\pm5.2\%$ after single-dose treatment, and the effect was sustained after long-term treatment ($9.0\pm3.7\%$), whereas flow-mediated dilation was $8.6\pm4.7\%$ at baseline and remained unchanged after single-dose ($7.8\pm4.4\%$) and long-term ($7.9\pm4.5\%$) treatment with placebo ($P=0.005$ by repeated-measures ANOVA). Plasma ascorbic acid concentrations increased from 41.4 ± 12.9 to 115.9 ± 34.2 μmol/L after single-dose treatment and to 95.0 ± 36.1 μmol/L after long-term treatment ($P<0.001$).

Conclusions—In patients with CAD, long-term ascorbic acid treatment has a sustained beneficial effect on EDNO action. Because endothelial dysfunction may contribute to the pathogenesis of cardiovascular events, this study indicates that ascorbic acid treatment may benefit patients with CAD. (***Circulation***. 1999;99:3234-3240.)

Key Words: coronary disease ■ endothelium ■ vitamins

Endothelium-derived nitric oxide (EDNO) plays a critical role in the regulation of vasomotor tone, platelet activity, and leukocyte adhesion to the vascular wall.[1] EDNO action is impaired in coronary artery disease (CAD) and may contribute to its clinical expression.[2] Recent studies suggest that increased vascular production of superoxide anion contributes to impaired EDNO action in atherosclerosis.[3] Another feature of atherosclerosis is vascular accumulation of oxidized LDL (ox-LDL), which is cytotoxic, inhibits NO release from endothelial cells, and may inactivate NO directly.[4]

A link between these abnormalities and impaired EDNO action is supported by studies demonstrating improved endothelium-dependent dilation after treatment with antioxidants, including ascorbic acid (vitamin C).[5–9] Ascorbic acid scavenges superoxide anion,[10] inhibits LDL oxidation,[11] and also plays an important role in the control of intracellular redox state.[12] We previously demonstrated that a single oral dose of ascorbic acid improves brachial artery flow-mediated dilation in patients with CAD.[5] Other investigators have also reported that short-term ascorbic acid treatment significantly improves EDNO-mediated vasodilation in forearm resistance vessels of patients with coronary risk factors[6,8,9] and the coronary arteries of patients with hypertension.[7]

Although the short-term effects of ascorbic acid on endothelial function are well established, the potential role of ascorbic acid for long-term therapy for CAD has not previously been examined. The purpose of this study was to determine whether long-term ascorbic acid supplementation is associated with sustained improvement in endothelial function and to gain insights into the mechanisms of that effect.

Methods

Research Subjects

Patients with angiographically documented CAD (≥1 stenosis $>50\%$) were eligible for study. Patients were excluded if they had unstable angina, acute myocardial infarction within 1 week, uncontrolled hypertension, congestive heart failure, or any other condition

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that would preclude safely withholding vasoactive medications. Other exclusion criteria included clinically significant valvular heart disease, use of antioxidant vitamins (vitamin C or E or β-carotene) within 1 month, initiation of cholesterol-lowering therapy within 6 months, and estrogen replacement therapy. All subjects provided written informed consent.

Study Protocol

Each subject made 2 visits 30 days apart. Subjects were instructed not to eat or drink on the morning of each visit and, if applicable, not to smoke for at least 24 hours. All medications were held for >12 hours, and all long-acting vasoactive medications were held for >24 hours before evaluation.

During the first visit, vital signs were recorded and fasting blood and urine samples were obtained. Flow-mediated dilation of the brachial artery was determined noninvasively with vascular ultrasound as previously described.[5,13] Hyperemia was induced by a cuff that was inflated on the proximal portion of the upper arm to occlude arterial flow (>200 mm Hg) for 5 minutes and then rapidly deflated. Ultrasound images of the bronchial artery were obtained at baseline and 1 minute after deflation.

In a randomized, double-blind fashion, subjects were then treated with ascorbic acid (2 g PO) or matched placebo tablets (Leiner Health Products). Two hours after treatment, vital signs were recorded, a blood sample was obtained, and the noninvasive assessment of flow-mediated dilation was repeated. After a 10-minute rest period, brachial images were obtained before and 3 minutes after sublingual nitroglycerin (0.4 mg). Nitroglycerin was omitted if the systolic blood pressure was <100 mm Hg or if the subject had previously had an adverse reaction to nitroglycerin.

Subjects continued their assigned treatment (ascorbic acid 500 mg/d or placebo) for the next month. They were instructed to take their study medication on the morning of the return visit, but all vasoactive medications were held as for the initial visit. Vital signs were recorded, blood and urine samples were collected, and the noninvasive assessment of flow-mediated dilation and nitroglycerin-mediated dilation were again performed.

Ultrasound images were digitized, and vessel diameter was measured by use of validated, customized software by personnel blinded to both image sequence and treatment assignment.[5] Maximal hyperemic flow was estimated by use of velocity-flow integrals and vessel cross-sectional area as previously described.[5]

Biochemical Analyses

Serum total cholesterol, triglycerides, HDL cholesterol, and glucose were determined with an automated analyzer (Hitachi Instruments). LDL cholesterol was calculated according to the Friedewald formula.[14] Ascorbic acid concentrations were determined in metaphosphoric acid–precipitated plasma that was stored at $-70°C$ as described.[11]

A blinded post hoc analysis was performed to explore the effect of treatment on markers of lipoprotein oxidation and cellular glutathione with consecutive samples selected at random from each treatment group without regard to clinical characteristics or vascular function. We determined plasma concentrations of 8-epi-prostaglandin $F_{2\alpha}$, a stable and specific marker of in vivo lipid peroxidation,[15] with an ELISA kit (Cayman Corp) and a previously described sample preparation protocol.[16]

o,o'-Dityrosine and o-tyrosine have been reported to be products of myeloperoxidase- and metal ion–catalyzed LDL oxidation, respectively.[17] Dityrosine levels are elevated in LDL isolated from atherosclerotic lesions.[17] We measured urine concentrations of these markers with gas chromatography with stable isotope dilution mass spectrometry. Urine samples (1 mL) were supplemented with 10% (vol/vol) trichloroacetic acid and centrifuged at 14 000 rpm for 5 minutes. Then 0.4 mL of urine supplemented with 100 μL of trichloroacetic acid and ^{13}C-labeled internal standards was loaded onto a C18 solid-phase extraction column (3 mL; Supelclean SPE; conditioned as described[17]). The column was washed with 6 mL of 0.1% trifluoroacetic acid. Amino acids were eluted with 3 mL of 10% methanol, concentrated to dryness under vacuum, and then derivatized for analysis.

TABLE 1. Clinical Characteristics

	Placebo	Ascorbic Acid
No.	25	21
Age, y	54±9	56±12
Male	25 (100)	17 (81)*
Prior myocardial infarction	17 (68)	16 (76)
Tobacco use, pack-y	40±29	33±33
Current tobacco use	6 (24)	5 (23)
Diabetes mellitus	3 (12)	7 (33)
Hypertension	20 (80)	13 (62)
Family history of premature CAD	10 (40)	7 (33)
Exercise (>3 times/wk)	15 (60)	14 (67)
Lipid-lowering agent	14 (56)	12 (57)
ACE inhibitor	7 (28)	5 (24)
Aspirin	25 (100)	21 (100)
Body weight, kg	89±14	87±17

Values are mean±SD or n (%).

*$P=0.03$ for sex. Diabetes mellitus was defined by history or fasting glucose >140 mg/dL; hypertension was defined by history or blood pressure >140/90 mm Hg.

To gain insight into the effect of ascorbic acid treatment on intracellular glutathione, leukocytes were isolated by density gradient centrifugation (1-Step, Accurate Chemical and Scientific Co) and precipitated with 5% metaphosphoric acid before storage at $-70°C$. Leukocyte glutathione concentration (oxidized plus reduced) was determined with a 5,5′-dithiobis(2-nitrobenzoid acid)-glutathione disulfide reductase kinetic assay as previously described.[18]

Statistical Analyses

Data are presented as mean±SD unless otherwise indicated. Clinical characteristics for the 2 treatment groups were compared by the unpaired t test or the χ^2 test as appropriate. The effect of treatment on brachial artery diameter, flow-mediated dilation, hyperemic flow, and the biochemical analyses listed above were compared by repeated-measures ANOVA with post hoc Student-Newman-Keuls comparison. Statistical analysis was performed with SigmaStat for Windows 2.03 (SPSS Inc).

Results

Subject Characteristics

Fifty-five subjects were enrolled. Eight were excluded before the data were unblinded because of poor image quality. One subject was excluded because his physician initiated therapy with an ACE inhibitor during the study. The clinical characteristics of the remaining 46 subjects are shown in Tables 1 and 2. There were 4 postmenopausal women in the ascorbic acid group and none in the placebo group ($P=0.03$). There was a trend toward lower fasting glucose concentration in the placebo group ($P=0.10$). Otherwise, there were no significant differences in baseline characteristics and no change in these characteristics during treatment.

Brachial Artery Responses

As shown in Figure 1, baseline brachial artery flow-mediated dilation was similar in the ascorbic acid (6.6±3.5%) and placebo (8.6±4.7%) groups ($P=0.11$). Flow-mediated dilation improved 2 hours after a single 2-g dose of ascorbic acid (10.1±5.2%, $P<0.05$). The improvement was sustained after

TABLE 2. Effect of Treatment on Fasting Serum Lipids and Glucose

	Placebo		Ascorbic Acid	
	Baseline	1 mo	Baseline	1 mo
Total cholesterol, mg/dL	212±31	203±20	207±46	204±50
HDL, mg/dL	40±11	44±15	39±9	39±6
LDL, mg/dL	133±30	128±26	123±33	117±30
Triglycerides, mg/dL	212±141	180±103	223±135	218±156
Glucose, mg/dL	108±53	101±17	126±51	135±66

All data are mean±SD. There were no significant differences between treatment groups or visits.

treatment for 30 days with 500 mg/d of ascorbic acid ($9.0\pm3.7\%$, $P<0.05$). In contrast, flow-mediated dilation was unchanged after single-dose ($7.8\pm4.4\%$) and long-term ($7.9\pm4.5\%$) administration of placebo. The response to ascorbic acid was significantly different from the response to placebo ($P=0.005$). As shown in Figure 1, there were no significant differences between the ascorbic acid and placebo groups in the response to sublingual nitroglycerin after single-dose ($13.0\pm7.6\%$ versus $11.8\pm4.5\%$, respectively) or long-term ($10.9\pm6.2\%$ versus $13.0\pm4.8\%$, respectively) treatment.

As shown in Table 3, there were no differences in baseline arterial diameter, hyperemia, blood pressure, or heart rate
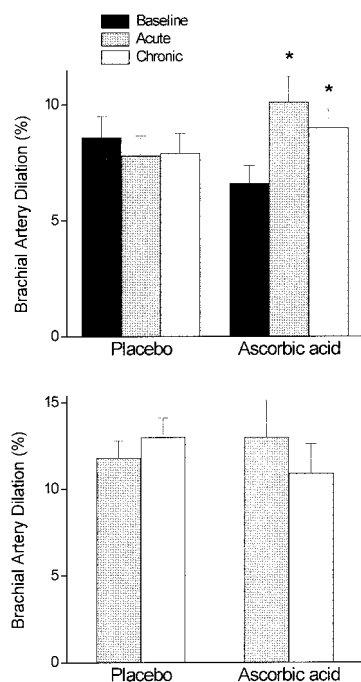

Figure 1. Effect of short-term and long-term oral ascorbic acid on flow-mediated dilation (top) or nitroglycerin-mediated dilation (bottom) of brachial artery in patients with CAD. Subjects underwent brachial ultrasound examination at baseline, 2 hours after a single 2-g oral dose of ascorbic acid, and after 1 month of oral 500 mg/d supplementation. Compared with placebo, ascorbic acid treatment improved flow-mediated dilation ($P=0.005$). Ascorbic acid had no effect on nitroglycerin-mediated dilation. Data are presented as mean±SEM and are derived from 21 subjects treated with ascorbic acid and 25 subjects treated with placebo.

between groups before or after treatment. There were no differences in the extent of improvement in diabetic subjects compared with nondiabetic subjects (data not shown). Ascorbic acid also improved endothelial function in both men and women and in subjects who were and were not receiving lipid-lowering therapy (data not shown).

Subjects With Abnormal Baseline Flow-Mediated Dilation

In our previous study, subjects with flow-mediated dilation <5% derived the most benefit from short-term ascorbic acid treatment.[5] In the present study, there were too few subjects in this category for meaningful analysis. Therefore, we examined the subset of 33 subjects (16 placebo, 17 ascorbic acid) with baseline flow-mediated dilation <10%, which represents the mean response for healthy individuals in our laboratory. As shown in Figure 2, flow-mediated dilation improved from $5.2\pm2.2\%$ to $8.8\pm5.0\%$ ($P<0.05$) after single-dose treatment and to $8.5\pm3.8\%$ ($P<0.05$) after long-term treatment. In contrast, there was no change in flow-mediated dilation from baseline ($6.2\pm2.3\%$) after single-dose ($5.9\pm2.9\%$) or long-term placebo treatment ($5.7\pm2.5\%$). The response to ascorbic acid was significantly different from the response to placebo ($P=0.002$). As shown in Figure 2, the response to nitroglycerin was similar in the ascorbic acid and placebo subsets of patients after single-dose treatment ($11.5\pm3.6\%$ and $11.1\pm6.0\%$, respectively) and long-term treatment ($9.2\pm4.3\%$ and $12.5\pm4.7\%$, respectively). Baseline vessel diameter, blood flow, hyperemic response, and hemodynamic parameters for the 2 groups were similar at baseline and after single-dose and long-term treatment. Subjects with baseline flow-mediated dilation >10% demonstrated no significant improvement after single-dose or long-term treatment (data not shown).

Ascorbic Acid, Total Glutathione, and Markers of Oxidation

As shown in Figure 3, plasma ascorbic acid concentrations at baseline were similar in the ascorbic acid and placebo groups (41 ± 13 and 43 ± 19 μmol/L, respectively). Plasma concentration increased to 116 ± 34 μmol/L ($P<0.05$) 2 hours after administration of 2 g of ascorbic acid and to 95 ± 36 μmol/L ($P<0.05$) after 30 days of supplementation with 500 mg/d. In the placebo group, the plasma ascorbic acid concentration was unchanged after single-dose (43 ± 20 μmol/L) and long-term (38 ± 20 μmol/L) treatment. By repeated-measures ANOVA, the response to ascorbic acid differed significantly from placebo ($P<0.001$).

Blood for ascorbic acid concentration was collected 2.6 ± 1.3 hours after the 500-mg dose of ascorbic acid on the day of the follow-up visit. In a subset of 5 subjects assigned to ascorbic acid treatment, blood was collected and the brachial ultrasound was performed at the time of trough rather than peak ascorbic acid level (24 hours after the dose). Plasma ascorbic acid in these subjects was 49 ± 16 μmol/L at baseline and 62 ± 19 μmol/L at 30 days ($P=0.08$), and flow-mediated dilation was $4.0\pm3.4\%$ at baseline and $8.4\pm4.3\%$ after 30 days of treatment ($P=0.009$). These results suggest that the observed improvement in flow-

TABLE 3.   Brachial Artery and Hemodynamic Parameters

Parameter	Placebo			Ascorbic Acid		
	Baseline	2 h	1 mo	Baseline	2 h	1 mo
Systolic blood pressure, mm Hg	132±18	135±17	139±22	145±26	140±23	142±26
Diastolic blood pressure, mm Hg	77±9	78±8	77±10	78±8	78±8	79±11
Heart rate, bpm	61±11	61±13	60±9	60±9	60±11	63±9
Artery diameter, mm	4.5±0.6	4.4±0.6	4.6±0.6	4.3±0.9	4.3±0.9	4.4±0.8
Hyperemic flow, % increase	750±340	880±310	750±260	630±310	860±440	720±380

Data are mean±SD. There were no significant differences in these parameters based on treatment, and no significant change was observed after treatment in either group by repeated-measures ANOVA.

mediated dilation in the whole group did not represent a short-term effect of the morning dose of ascorbic acid.

We also examined the effects of long-term ascorbic acid treatment on markers of lipid and protein oxidation and leukocyte glutathione levels in subsets of subjects. As shown in Table 4, there was no evidence that long-term ascorbic acid treatment reduced plasma 8-epi-prostaglandin $F_{2\alpha}$ concentration, urinary o,o'-dityrosine, or o-tyrosine and no evidence that treatment increased leukocyte total glutathione concentration. The study had 80% power ($\alpha=0.05$) to detect a change of 46% for 8-epi-prostaglandin $F_{2\alpha}$, 21% for o,o'-dityrosine, and 50% for leukocyte glutathione.
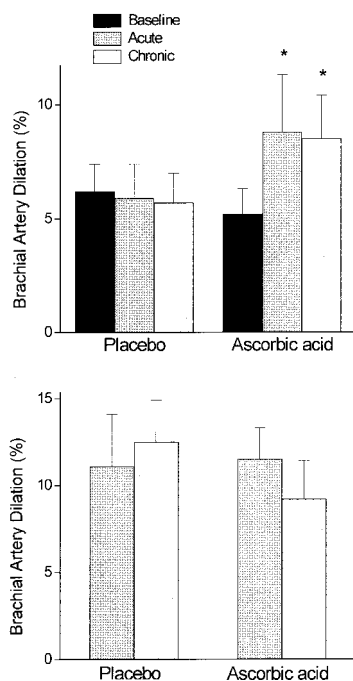

Figure 2. Effect of short-term and long-term oral ascorbic acid on flow-mediated dilation (top) or nitroglycerin-mediated dilation (bottom) of brachial artery in patients with CAD and baseline flow-mediated dilation <10%. Subjects underwent brachial ultrasound examination at baseline, 2 hours after a single 2-g oral dose of ascorbic acid, and after 1 month of oral 500 mg/d supplementation. Compared with placebo, ascorbic acid improved flow-mediated dilation ($P=0.002$). Ascorbic acid had no effect on nitroglycerin-mediated dilation. Data are presented as mean±SEM and are derived from 17 subjects treated with ascorbic acid and 16 subjects treated with placebo.

Discussion

This randomized, double-blind, placebo-controlled study demonstrates that a single oral dose of ascorbic acid improves brachial artery flow-mediated dilation in patients with angiographically proven CAD. This beneficial effect is sustained after 1 month of ascorbic acid treatment. The improvement is particularly notable in subjects with less than normal baseline flow-mediated dilation. The effect on vasomotor function could not be attributed to an increase in the stimulus for dilation (hyperemia) or a change in the capacity of the artery to dilate to an exogenous source of NO (nitroglycerin). Thus, the study suggests that single-dose and long-term ascorbic acid treatments improve the production or bioactivity of EDNO in the brachial artery of patients with CAD.

The findings of the present study confirm the findings of our previous study in patients with CAD[5] and extend those findings to long-term treatment. A similar effect of both single-dose and long-term (2 g/d for 1 month) ascorbic acid treatment was recently described in the radial arteries of patients with congestive heart failure.[19] Those improvements were reversed by intra-arterial N^G-monomethyl-L-arginine infusion, suggesting a dependence on NO synthesis.[19] Recent studies have also demonstrated improved endothelium-dependent vasodilation after the short-term administration of
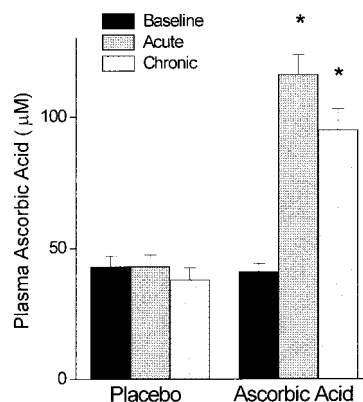

Figure 3. Effect of ascorbic acid treatment on plasma ascorbic acid concentration in patients with CAD. Plasma ascorbic acid concentration was determined at baseline, 2 hours after a single 2-g oral dose of ascorbic acid, and after 1 month of oral 500 mg/d supplementation. Compared with placebo, ascorbic acid increased plasma ascorbic acid concentration ($P=0.001$). Data are presented as mean±SEM and are derived from 21 subjects treated with ascorbic acid and 25 subjects treated with placebo.

TABLE 4. Effect of Treatment on Markers of Oxidation and Leukocyte Glutathione

	Placebo		Ascorbic Acid	
	Baseline	1 mo	Baseline	1 mo
Plasma 8-epi-prostaglandin $F_{2\alpha}$, pg/mL	85±30 (7)	72±24 (7)	59±27 (8)	81±28 (8)
Urine o,o'-dityrosine, pmol/nmol creatinine	5.8±1.3 (6)	6.2±1.9 (6)	5.6±1.7 (16)	5.7±1.6 (16)
Urine o-tyrosine, pmol/nmol creatinine	4.8±2.4 (6)	6.1±1.0 (6)	6.8±3.2 (16)	6.9±2.7 (16)
Leukocyte glutathione, μmol/g protein	6.8±2.9 (9)	6.4±2.4 (9)	7.6±4.1 (7)	6.7±1.9 (7)

All data are mean±SD (n). There were no significant differences between treatment groups or visits. Numbers in parentheses indicate number of subjects in each group.

ascorbic acid in patients with hypertension,[7] adult-onset diabetes mellitus,[6] or cigarette smoking[8] or after a high-fat meal.[20] One previous study failed to demonstrate improvement after long-term treatment with a combination of vitamin E, β-carotene, and ascorbic acid (1 g/d for 1 month) in forearm resistance vessels of patients with hypercholesterolemia.[21] This apparently discordant finding may reflect differences in patient population and concurrent therapy and/or differential responses of conduit and resistance vessels.

With regard to mechanism, it has been proposed that ascorbic acid improves EDNO action by scavenging superoxide anion and preventing "inactivation" of NO.[5,6] This explanation is attractive because atherosclerosis and hypercholesterolemia are linked to excess generation of superoxide, and superoxide reacts with NO and eliminates its biological activity.[3] One caveat lies in the estimation that only supraphysiological concentrations of ascorbic acid could make this mechanism relevant in vivo. Although ascorbic acid scavenges superoxide, the bimolecular rate constant for this reaction (3.3×10^5 mol/$L^{-1} \cdot s^{-1}$) is ≈10^5 times less than the rate constant for the reaction between superoxide and NO (1.9×10^{10} mol/$L^{-1} \cdot s^{-1}$).[10] Assuming an NO concentration of 0.1 to 1 μmol/L adjacent to the endothelial surface,[22] one would predict that an ascorbic acid concentration of 10 to 100 mmol/L would be required to preserve EDNO action. We recently confirmed this prediction using an in vitro model of superoxide-induced endothelial dysfunction.[10] Thus, it is unlikely that extracellular superoxide scavenging is responsible for the observed effects on endothelial function in the present study, in which the peak plasma ascorbic acid concentration was 116 μmol/L. However, it remains possible that this mechanism was operative in previous studies in which intra-arterial infusion produced plasma concentrations of 1 to 10 mmol/L.[6,8]

Another potential mechanism for the effect of ascorbic acid on endothelial function is inhibition of LDL oxidation.[11] ox-LDL is cytotoxic to endothelial cells and may inactivate NO directly.[4] Moreover, ox-LDL inhibits receptor-dependent NO release from endothelial cells, an effect that is attributable to formation of lysophosphatidylcholine and altered cell membrane signal transduction.[23] In the present study, we found that long-term ascorbic acid treatment did not reduce plasma 8-epi-prostaglandin $F_{2\alpha}$ or urine o,o'-dityrosine concentration. As noted above, the study had sufficient power to detect changes of 46% and 21%, respectively, in these markers of lipid and protein oxidation. This power compares favorably with available data. For example, smoking cessa-

tion for 2 weeks is associated with a 38% decrease in plasma F_2 isoprostanes.[16] In a preliminary study, we recently demonstrated a 13% reduction in o,o'-dityrosine after α-tocopherol treatment of healthy adults.[24] Thus, despite the limited sample sizes, we could have detected changes in these markers comparable to those of published data. Clearly, it remains possible that smaller effects were missed or that these systemic markers do not fully reflect events in the vascular wall.

In addition to scavenging superoxide anion or inhibiting LDL oxidation, ascorbic acid could alternatively improve EDNO action by sparing intracellular glutathione, which together with ascorbic acid is the primary regulator of intracellular redox state.[12] A previous study suggested that ascorbic acid 500 mg/d for 2 weeks increased red blood cell glutathione by 47% in healthy subjects.[25] In support of the importance of glutathione for EDNO action, we recently demonstrated that augmenting intracellular glutathione improves brachial artery flow-mediated dilation in patients with CAD.[13] Our present study failed to show an increase in leukocyte total glutathione concentration after ascorbic acid treatment, which argues against this potential mechanism. However, consideration should also be given to the possibility that ascorbic acid increased the ratio of reduced to oxidized glutathione rather than total glutathione, although an investigation of this possibility would pose logistic problems that were beyond the scope of this human study. Another consideration is that leukocyte glutathione levels may not accurately reflect the redox status of the vascular wall.

Our finding that long-term ascorbic acid treatment improves flow-mediated dilation may be clinically relevant, because other interventions that improve endothelial function have been linked to a reduction in cardiovascular events.[1,2] There is growing evidence that antioxidant status is relevant to the clinical expression of CAD.[4] With regard to ascorbic acid, leukocyte concentrations are significantly reduced in patients with angiographically documented CAD compared with normal subjects.[26] We recently demonstrated that low plasma ascorbic acid levels are associated with the presence of an unstable coronary syndrome.[27] Previous studies have suggested that ascorbic acid may reduce cardiovascular risk by lowering blood pressure[28] or raising HDL cholesterol[29]; however, our randomized, double-blind, placebo-controlled study demonstrated no such effects in patients with CAD. In a population study, low dietary ascorbic acid intake (<50 mg/d) was associated with increased cardiovascular risk.[30] Conversely, other studies[31,32] have shown no benefit with

increased ascorbic acid intake. Such negative findings have been explained by the failure to consider a sufficiently low intake level, because tissue stores become saturated with an intake of only 100 mg/d.[33] It remains unknown whether ascorbic acid treatment will reduce cardiovascular risk in CAD patients, but the present study suggests that the beneficial effects of such treatment might occur because of improved endothelial function.

The present study has several limitations. First, we examined the brachial artery, and inferences about the coronary circulation must be made with caution. However, a recent study suggested a close relationship between endothelial dysfunction in these 2 vascular beds.[34] Second, on the return visit, we examined brachial artery flow-mediated dilation at the time of peak ascorbic acid concentration.[5] Although we also observed a beneficial effect at the time of trough level in a small subgroup, we do not have such data for the entire group. It is notable that Hornig and colleagues[19] also showed improved endothelial vasomotor function after long-term therapy both before and after a dose of ascorbic acid in patients with congestive heart failure. Third, a number of these subjects with CAD had relatively normal endothelial vasomotor function and would be expected to have little response to an intervention. In addition, there was a nonsignificant trend toward higher flow-mediated dilation in the placebo group. We addressed these issues by examining the subgroup of patients with baseline flow-mediated dilation $<10\%$. In that subgroup, baseline responses were more similar, and as for the group as a whole, ascorbic acid had a highly significant effect. Fourth, although our study was randomized and double-blind, we acknowledge the possibility that some unmeasured confounding variable could have influenced the results. Finally, our exploratory investigation of the effect of ascorbic acid on LDL oxidation was limited by the small sample size and focused on systemic (rather than vascular tissue) markers.

In summary, this randomized, double-blind, placebo-controlled study demonstrates that both single-dose and long-term treatment with ascorbic acid improves endothelial vasomotor function in the brachial artery of patients with CAD. The improvement in function was accompanied by a significant rise in plasma ascorbic acid concentration and a lack of change in blood pressure, lipoprotein profile, or systemic markers of oxidative damage. We speculate that ascorbic acid may improve vasomotor function by enhancing the intracellular redox state or by some other undetermined mechanism. The sustained improvement in endothelial function may, in part, explain the observed epidemiological findings linking ascorbic acid intake with reduction in risk of ischemic heart disease. These results provide further justification for trials investigating ascorbic acid therapy and cardiovascular disease.

Acknowledgments

References

1. Gokce N, Keaney JF Jr, Vita JA. Endotheliopathies: clinical manifestations of endothelial dysfunction. In: Loscalzo J, Shafer AI, eds. *Thrombosis and Hemorrhage*. 2nd ed. Baltimore, Md: Williams & Wilkins; 1998:901–924.
2. Levine GN, Keaney JF Jr, Vita JA. Cholesterol reduction in cardiovascular disease: clinical benefits and possible mechanisms. *N Engl J Med*. 1995;332:512–521.
3. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest*. 1993;91:2546–2551.
4. Diaz MN, Frei B, Vita JA, Keaney JF Jr. Antioxidants and atherosclerotic heart disease. *N Engl J Med*. 1997;337:408–417.
5. Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney JF Jr, Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation*. 1996;96:1107–1113.
6. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest*. 1996;97:22–28.
7. Solzbach U, Hornig B, Jeserich M, Just H. Vitamin C improves endothelial dysfunction of epicardial coronary arteries in hypertensive patients. *Circulation*. 1997;96:1513–1519.
8. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation*. 1996;94:6–9.
9. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation*. 1997;97:2222–2229.
10. Jackson TS, Xu A, Vita JA, Keaney JF Jr. Ascorbate prevents the interaction of superoxide and nitric oxide only at high physiological concentrations. *Circ Res*. 1998;83:916–922.
11. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci U S A*. 1989;86:6377–6381.
12. Meister A. Glutathione-ascorbic acid antioxidant system in animals. *J Biol Chem*. 1994;269:9397–9400.
13. Vita JA, Frei B, Holbrook M, Gokce N, Leaf C, Keaney JF Jr. L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J Clin Invest*. 1998;101:1408–1414.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
15. Longmire AW, Sift LL, Roberts LJ II, Awad JA, Burk RF, Morrow JD. Effect of oxygen tension on the generation of F2-isoprostanes and malondialdehyde in peroxidizing rat liver microsomes. *Biochem Pharmacol*. 1994;47:1173–1177.
16. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ. Increase in circulating products of lipid peroxidation (F2 isoprostanes) in smokers: smoking as a cause of oxidative damage. *N Engl J Med*. 1995;332:1198–1203.
17. Leeuwenburgh C, Rasmussen JE, Hsu FF, Mueller DM, Pennathur S, Heinecke JW. Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *J Biol Chem*. 1997;272:3520–3526.
18. Ackerboom TPM, Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol*. 1981;77:373–382.
19. Hornig B, Arakawa N, Kohler C, Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation*. 1998;97:363–368.
20. Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *JAMA*. 1997;278:1682–1686.
21. Gilligan DM, Sack MN, Guetta V, Casino PR, Quyyumi AA, Rader DJ, Panza JA, Cannon RO. Effect of antioxidant vitamins on low density lipoprotein oxidation and impaired endothelium-dependent vasodilation in patients with hypercholesterolemia. *J Am Coll Cardiol*. 1994;24:1611–1617.

22. Malinski T, Taha Z, Grunfeld S, Patton S, Kapturczak M, Tomboulian P. Diffusion of nitric oxide in the aorta wall monitored in situ by porphyrinic microsensors. *Biochem Biophys Res Commun*. 1993;193: 1076–1082.

23. Kugiyama K, Kerns SA, Morrisett JD, Roberts R, Henry PD. Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. *Nature*. 1990;344:160–162.

24. Dillon G, Vita JA, Leeuwenburgh C, Olesiak M, Heinecke JW, Frei B. α-Tocopherol supplementation reduces systemic markers of oxidative damage in healthy adults. *Circulation*. 1998;98(suppl I):I-671.

25. Johnston CS, Meyer C, Srilakshmi JC. Vitamin C elevates red blood cell glutathione in healthy adults. *Am J Clin Nutr*. 1993;58:103–105.

26. Ramirez J, Flowers NC. Leukocyte ascorbic acid and its relationship to coronary artery disease in man. *Am J Clin Nutr*. 1980;33:2079–2087.

27. Vita JA, Keaney JF Jr, Raby KE, Morrow JD, Freedman JE, Lynch S, Koulouris SN, Hankin BR, Frei B. Low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome. *J Am Coll Cardiol*. 1998;31:980–986.

28. Ceriello A, Giugliano D, Quatraro A, Lefebvre P. Anti-oxidants show an anti-hypertensive effect in diabetic and hypertensive subjects. *Clin Sci*. 1991;81:739–742.

29. Hallfrisch J, Singh VN, Muller DC, Baldwin H, Bannon ME, Andres R. High plasma vitamin C associated with high plasma HDL- and HDL2 cholesterol. *Am J Clin Nutr*. 1994;60:100–105.

30. Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology*. 1992;3:194–202.

31. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*. 1993;328:1450–1456.

32. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med*. 1993;328:1444–1449.

33. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci U S A*. 1996;93: 3704–3709.

34. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Leiberman E, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.