Ascorbate Prevents the Interaction of Superoxide and Nitric Oxide Only at Very High Physiological Concentrations

Terence S. Jackson, Aiming Xu, Joseph A. Vita, John F. Keaney, Jr

Abstract—The bioactivity of nitric oxide (*NO) depends, in part, on its interaction with superoxide. Usually, superoxide dismutase (SOD) preserves *NO bioactivity by limiting the availability of superoxide. Ascorbic acid also effectively scavenges superoxide, but the extent to which this interaction is necessary for intact *NO bioactivity is not known. Therefore, the present study examined the effect of ascorbic acid on *NO bioactivity with isolated rabbit arterial segments. A steady flux of superoxide (1.15 to 2.3 μmol L⁻¹ min⁻¹) produced either by pyrogallol autoxidation or a hypoxanthine/xanthine oxidase system inhibited endothelium-derived *NO-mediated arterial relaxation elicited by acetycholine. This effect of superoxide was completely blocked by SOD (300 IU/mL) and the manganese SOD mimic EUK-8 (300 μmol/L) and partially inhibited by ascorbic acid (10 mmol/L). Lower concentrations of ascorbic acid were ineffective despite scavenging >90% of superoxide. We increased the endogenous flux of superoxide (3.2±0.3-fold) by inhibiting vascular copper-zinc SOD with diethyldithiocarbamate. This increased endogenous flux of superoxide produced an impairment of *NO-mediated arterial relaxation that was reversed by EUK-8 (300 μmol/L) but not ascorbic acid (10 mmol/L) despite equivalent scavenging of the endogenous superoxide flux. We used 3-nitrotyrosine formation (from peroxynitrite) as an indicator of *NO interaction with superoxide and found that SOD and EUK-8 compete more effectively with *NO for superoxide than does ascorbic acid. These data indicate that preservation of *NO bioactivity by superoxide scavengers depends not only on superoxide scavenging activity, but also on the rate of superoxide scavenging. Normal extracellular concentrations of ascorbic acid (30 to 150 μmol/L) are not likely to prevent the interaction of *NO with superoxide under physiological conditions. (Circ Res. 1998;83:916-922.)

Key Words: antioxidant ■ free radical ■ blood vessel ■ oxidant ■ peroxynitrite

Normal vascular homeostasis depends on endothelial elaboration of paracrine factors that prevent both platelet adhesion to the endothelial surface and inappropriate vasospasm. One important endothelial product responsible for these functions is nitric oxide (*NO), a free radical produced constitutively by the vascular endothelium. Abnormalities in *NO action and metabolism are known to develop in association with vascular disease and have been implicated in the development of clinically significant vascular events.¹

In vivo, *NO is subject to rapid inactivation by the superoxide anion,²–⁴ an obligate product of normal oxidative metabolism.¹⁵ Endothelial cells constitutively produce both superoxide⁶ and *NO,⁷ suggesting that the effective release of *NO from the vascular endothelium depends on the relative concentrations of these 2 species.

Usually, the availability of superoxide in tissues is strictly limited by the abundant tissue concentration of superoxide dismutase (SOD) that may approach 10 μmol/L.² However, superoxide and *NO react rapidly with a bimolecular rate constant that approaches the diffusion limit (1.9×10¹⁰ mol · L⁻¹ · s⁻¹)⁹ and is similar to the rate of superoxide dismutation by SOD (2×10⁹ mol · L⁻¹ · s⁻¹).⁸ These data indicate that *NO competes effectively with SOD for superoxide. Considerable data now exist to support this position. For example, inhibition of endothelial cell copper-zinc SOD impairs effective release of *NO from endothelial cells.¹⁰,¹¹ Intact copper-zinc SOD function is also required for smooth muscle cell relaxation in response to nitrovasodilators.¹² In addition, abnormalities in *NO-mediated arterial relaxation associated with hypercholesterolemia,¹²,¹³ diabetes mellitus,¹⁴ and hypertension¹⁵ have been linked to excess vascular levels of superoxide. Thus, *NO-mediated arterial relaxation depends on SOD activity to limit the availability of superoxide.

The tissue availability of superoxide is limited by its interaction with other compounds in addition to SOD. Antioxidants such as α-tocopherol,¹⁶ glutathione,¹⁷ and ascorbic acid¹⁸ are known to react with superoxide. Recent studies have demonstrated that acute treatment with ascorbic acid improves *NO-mediated arterial relaxation in patients with atherosclerosis,¹⁹ but the mechanism of this effect is not clear. The action and metabolism of endothelium-derived *NO (EDNO) depends on vascular levels of superoxide²¹,¹²,¹₃; thus, it is conceivable that ascorbic acid may exert some control over *NO-mediated arterial relaxation by preventing the interaction of *NO and superoxide. The purpose of the present study was to determine the extent to which physio-
logically relevant concentrations of ascorbic acid influence arterial relaxation in response to EDNO.

**Materials and Methods**

Sodium pentobarbital was purchased from Anthony Products Co. Peroxynitrite was obtained from Alexis Corporation and diethylamine (DEA)-NO was purchased from Cayman Chemical Corp. Xanthine oxidase was purchased from Boehringer-Mannheim, Acetylcysteine hydrochloride, phenylephrine, diethylenetriamine pentaacetic acid (DTPA), SOD (copper-zinc form from bovine erythrocyte, 2500 to 7000 IU/mg), and all other compounds were purchased from Sigma Chemical Co.

Physiological salt solution (PSS) contained 118.3 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L MgSO4, 1.2 mmol/L KH2PO4, 25 mmol/L NaHCO3, 11.1 mmol/L glucose, 10 mmol/L indomethacin, and 0.026 mmol/L Na2EDTA. PBS consisted of 10 mmol/L NaH2PO4 and 0.15 mol/L NaCl (pH 7.4).

**In Vitro Assay of Vascular Function**

New Zealand White rabbits (2.5 to 3.5 kg) of either sex were used for the present study (Pine Acres Rabbitry, Vt). Animals consumed food and water ad libitum, and all animal studies were approved by the Boston University Medical Center Institutional Animal Care and Use Committee. The thoracic aorta was isolated from New Zealand White rabbits killed with pentobarbital (120 mg/kg) via a marginal ear vein. Vessel segments were prepared, suspended in organ chambers as previously described, and gassed with 95% O2/5% CO2. After equilibration for 90 minutes, vessels were contracted with phenylephrine (1 μmol/L) and vascular tone was assayed in response to the addition of acetylcholine or the endothelium-independent, cGMP-dependent vasodilator, atrial natriuretic peptide (ANP). When pyrogallol was used as a source of superoxide it was added 1 minute before the assessment of vascular function. In some experiments, hypoxantheme (100 μmol/L) was added to the PSS and superoxide generation initiated with 0.02 IU/mL xanthine oxidase 1 minute before assessing vascular function. Ascorbic acid was dissolved in PSS and the pH adjusted with NaOH to produce a final pH of 7.4 in the organ chamber. Ascorbic acid was added to organ chambers 10 minutes before the assessment of arterial relaxation.

In some studies, vessels were treated for 30 minutes with 5 mmol/L diethylidithiocarbamate (DTC) to inhibit copper-zinc SOD. Vessels treated with DTC were subsequently washed 3 times with PSS containing 100 μmol/L DTPA to remove any residual redox-active copper liberated by DTC treatment.

**Quantification of Superoxide and *NO**

The flux of superoxide from pyrogallol autoxidation was quantified as the reduction of cytochrome c inhibited by SOD with an extinction coefficient of 2.1×104 mol·L⁻¹·cm⁻¹ at 560 nm. The flux of *NO from DEA-NO decomposition was estimated spectrophotometrically at 250 nm (ε=6500 mol·L⁻¹·cm⁻¹) noting that each mole of DEA-NO produces ~1.5 mol *NO. Because ascorbate directly reduces cytochrome c, scavenging of superoxide by ascorbate was estimated by the inhibition of pyrogallol autoxidation, which is superoxide-dependent at pH 9.5. Pyrogallol (200 μmol/L) was incubated in PBS, and autoxidation was estimated by monitoring the change in absorbance at 420 nm with and without ascorbic acid or SOD. All additions of ascorbic acid were adjusted to achieve a final pH of 7.4.

**Vascular SOD Activity**

Segments of thoracic aorta were isolated as described above and incubated with PSS at 37°C gently bubbled with 95% O2/5% CO2. After 30 minutes, vessels were incubated for 10 minutes in 5-mL polyethylene tubes containing HEPES-buffered PSS (PSS containing 20 mmol/L HEPES) with 0.25 mmol/L lucigenin (bis-N-methylacridinium nitrate). After equilibration with lucigenin, vascular superoxide levels were estimated from chemiluminescence recorded with a Turner Designs Model 20e luminometer at 37°C in a dark, light-sealed room. The integral of the chemiluminescence signal was recorded at 30-second intervals for 5 minutes, and the integral readings were combined. Background chemiluminescence was determined from identically processed vessel-free incubations and subtracted from the determinations with vessels. Chemiluminescence was converted to superoxide by a standard curve relating known quantities of superoxide (from a xanthine/xanthine oxidase system as determined by SOD-inhibited cytochrome c reduction) to chemiluminescence. To inactivate copper-zinc SOD, vessels were incubated for 30 minutes with 5 mmol/L DDC, washed 3 times (10 mL with HEPES-buffered PSS containing DTPA (100 μmol/L), and superoxide determined as above. The effect of ascorbic acid, SOD, and EUK-8 (a cell-permeable manganese SOD [MnSOD] mimic; Evkaryon, Bedford, Mass) on vascular superoxide scavenging activity was determined by adding these compounds directly to the chemiluminescence chamber and repeating the measurement. In the absence of such additions, the chemiluminescence signal was stable during the time of the assay.

**Estimation of Peroxynitrite Formation**

The interaction of *NO and superoxide results in the formation of peroxynitrite that, in the presence of CO2, spontaneously reacts with tyrosine to form 3-nitrotyrosine. We estimated peroxynitrite formation as the production of 3-nitrotyrosine with a modification of the method described by van der Vliet et al.29 d,l-Tyrosine (1 mmol/L) in 10 mmol/L phosphate buffer with 50 μmol/L DTPA was incubated with DEA-NO (20 μmol/L) and pyrogallol (200 μmol/L) with or without ascorbic acid (0 to 10 mmol/L), SOD (0.3 to 300 IU/mL), or EUK-8 (0.1 to 300 μmol/L) for 15 minutes. The formation of 3-nitrotyrosine was analyzed by UV detection at 274 nm after separation on an LC-18 column (25 cm×4.6 mm, Supelco) with a mobile phase of 50 mmol/L KH2PO4, pH 3 and methanol (92:8).29 Ascorbic acid (0 to 10 mmol/L) had no effect on the yield of 3-nitrotyrosine when authentic peroxynitrite (10 mmol/L) was added to 2 mmol/L d,l-tyrosine (data not shown).

**Data Analysis**

All values are presented as mean±SEM. The vascular responses to acetylcholine and NO are reported as the percent reduction in tension (relaxation) compared with the contraction produced by 1 μmol/L phenylephrine. Dose responses to acetylcholine and ANP were compared within treatment groups with repeated-measures ANOVA and responses between treatment groups were compared with 2-way ANOVA with a post hoc Dunn’s or Dunnett’s test as appropriate. Statistical significance was accepted if the null hypothesis was rejected with a P<0.05.

**Results**

**Effect of Superoxide and Ascorbic Acid on Arterial Relaxation**

We observed dose-dependent arterial relaxation of the thoracic aorta in response to acetylcholine between the concentrations of 1 mmol/L and 10 μmol/L (Figure 1A). In contrast, superoxide (2.3±0.04 μmol·L⁻¹·min⁻¹) generated from autoxidation of pyrogallol (200 μmol/L) significantly inhibited the dose-dependent relaxation to acetylcholine with maximal relaxation reduced from 73±3% to 48±3% (P<0.05 by 2-way repeated-measures ANOVA). This effect of superoxide was completely reversed with 300 IU/mL SOD producing a maximal relaxation of 66±6% (P=NS versus control). To test the effect of superoxide on smooth muscle cell relaxation independent of *NO, we examined arterial relaxation in response to the cGMP-dependent vasodilator, ANP. As shown in Figure 1B, superoxide generated from pyrogallol (200 μmol/L) autoxidation had no effect on cGMP-dependent arterial relaxation in response to ANP.
To determine whether ascorbic acid can prevent the inactivation of \( \cdot \text{NO} \) by superoxide, we incubated arterial segments with pyrogallol and increasing doses of ascorbic acid just before the assessment of arterial relaxation. As expected, a steady flux of superoxide from pyrogallol autoxidation produced significant inhibition of EDNO-mediated arterial relaxation \( (P<0.05\hspace{1em}\text{by 2-way repeated-measures ANOVA; Figure } 2) \). The impairment of EDNO-mediated arterial relaxation by superoxide was prevented by ascorbic acid only at a concentration of 10 mmol/L (Figure 2A). We also sought to confirm these observations with another superoxide-generating system. We used a steady flux of superoxide \( (1.15\pm0.2\hspace{1em}\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}; \text{n}=3) \) from hypoxanthine \( (100\hspace{1em}\text{mmol/L}) \) and xanthine oxidase \( (0.02\hspace{1em}\text{IU/mL}) \) and observed significant inhibition of EDNO-mediated arterial relaxation that was reversed by SOD \( (P<0.05\hspace{1em}\text{by 2-way repeated-measures ANOVA; Figure } 2) \). Similar to the situation with pyrogallol, this impairment of EDNO-mediated arterial relaxation by superoxide was only partially prevented by ascorbic acid at a concentration of 10 mmol/L (Figure 2B).

Ascorbate and Superoxide Scavenging
To quantify the extent of superoxide scavenging by ascorbate and SOD in our system, we determined the inhibition of pyrogallol autoxidation, which is superoxide-dependent at pH<9.5. As presented in Figure 3, the autoxidation of pyrogallol was inhibited 91\% by 300 IU/mL SOD \( (P<0.05\hspace{1em}\text{versus control, } \text{n}=4) \) compared with 85\%, 99\%, 4\%, and 98\% by 0.05, 0.1, 1, and 10 mmol/L ascorbic acid, respectively (all \( P<0.05\hspace{1em}\text{versus control by 1-way ANOVA, } \text{n}=4) \). Thus, ascorbate effectively scavenges superoxide at concentrations that are considerably lower than those needed to preserve \( \cdot \text{NO} \)-mediated arterial relaxation.

Pyrogallol and Endothelial Cell Toxicity
To determine whether pyrogallol produced endothelial damage, we assessed acetylcholine-stimulated EDNO-mediated arterial relaxation in vessel segments before and after a 20-minute exposure to a flux of superoxide \( (2.3\hspace{1em}\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} ) \) from pyrogallol \( (200\hspace{1em}\text{mmol/L}) \) autoxidation. As shown in Figure 4, endothelium-dependent arterial relaxation to

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**Figure 1.** The effect of superoxide on \( \cdot \text{NO} \)-dependent arterial relaxation. Segments of thoracic aorta were harvested from New Zealand White rabbits as described in Materials and Methods. A, Vessels were contracted with phenylephrine (1 mmol/L) and relaxation assayed in response to the indicated concentrations of acetylcholine in the presence of PSS containing no additions \( (\bullet) \), 200 mmol/L pyrogallol \( (\square) \), 200 mmol/L pyrogallol with 300 IU/mL SOD \( (\blacklozenge) \), or 300 IU/mL SOD \( (\blacklozenge) \). Data represent mean±SEM of 5 to 8 experiments. \( *P<0.05\hspace{1em}\text{vs control group by 2-way ANOVA.} \)

**Figure 2.** Ascorbic acid and the impairment of \( \cdot \text{NO} \)-mediated arterial relaxation from superoxide. Segments of thoracic aorta were harvested from New Zealand White rabbits as described in Materials and Methods. A, Vessels were contracted with phenylephrine (1 mmol/L) and relaxation assayed in response to the indicated concentrations of acetylcholine in the presence of PSS containing no additions \( (\blacklozenge) \), 200 mmol/L pyrogallol \( (\square) \), or 200 mmol/L pyrogallol with 0.1 \( (\Delta) \), 1.0 \( (\bullet) \), or 10 \( (\bigtriangledown) \) mmol/L ascorbic acid. B, Vessels were prepared as in panel A, except relaxation was assessed in the presence of PSS containing no additions \( (\blacklozenge) \), 100 mmol/L hypoxanthine with 0.02 IU/mL xanthine oxidase \( (\bigtriangleup) \), or hypoxanthine with 0.1 \( (\Delta) \), 1.0 \( (\bullet) \), or 10 \( (\bigtriangledown) \) mmol/L ascorbic acid. Values are plotted as mean±SEM and are derived from 5 to 7 experiments in each group. \( *P<0.05\hspace{1em}\text{vs PSS alone or } \bigtriangledown P<0.05\hspace{1em}\text{vs } \text{HX/XO alone by 2-way ANOVA.} \)

**Figure 3.** Superoxide scavenging by SOD and ascorbic acid. Pyrogallol \( (200\hspace{1em}\text{mmol/L}) \) was incubated in PBS with or without the addition of SOD \( (300\hspace{1em}\text{IU/mL}) \) or the indicated concentrations of ascorbic acid. Pyrogallol autoxidation was estimated by the change in absorbance at 420 nm during a 10-minute incubation. Data are mean±SEM of 4 experiments. \( *P<0.05\hspace{1em}\text{vs no additions.} \)

**Figure 4.** Arterial relaxation before and after exposure to superoxide. Segments of thoracic aorta were harvested from New Zealand White rabbits as described in Materials and Methods. After contraction with phenylephrine (1 mmol/L) arterial relaxation was assayed in response to acetylcholine before \( (\blacktriangleleft) \), during \( (\blacktriangledown) \), and after \( (\blacklozenge) \) exposure to a flux of superoxide \( (2.3\hspace{1em}\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} ) \) caused by pyrogallol \( (200\hspace{1em}\text{mmol/L}) \) autoxidation. Values are plotted as mean±SEM and are derived from 6 experiments. \( *P<0.001\hspace{1em}\text{vs before pyrogallol by 2-way repeated-measures ANOVA.} \)
acetylcholine was the same before and after exposure to pyrogallol, indicating that short-term exposure to superoxide does not result in any permanent impairment in *NO-mediated arterial relaxation.

**Effect of Ascorbic Acid on *NO-Mediated Arterial Relaxation With an Endogenous Flux of Superoxide**

It is difficult to extrapolate observations with pyrogallol to events that are relevant in vivo. To generate a more relevant superoxide flux, we treated aortic segments with 5 mmol/L DDC, a copper chelator that inactivates endogenous copper-zinc SOD. As shown in Figure 5A, treatment of aortic segments with DDC produces a significant impairment in EDNO-mediated arterial relaxation in response to acetylcholine (P<0.001 versus no DDC by 2-way ANOVA). This impairment in EDNO-mediated arterial relaxation was not mitigated by ascorbic acid in concentrations up to 10 mmol/L (all P>0.05 versus no DDC by 2-way ANOVA; Figure 5A).

Authentic SOD (300 IU/mL) only partially restored EDNO-mediated arterial relaxation (Figure 5B), which was likely a result of its limited cellular access. In contrast, the MnSOD mimic EUK-8 (300 μmol/L) completely restored EDNO-mediated arterial relaxation in response to acetylcholine (Figure 5B). Endothelium-independent arterial relaxation to ANP was not impaired in DDC-treated vessels (Figure 5C).

**Vascular Superoxide Scavenging With Endogenous Superoxide**

To determine the extent of superoxide scavenging with an endogenous flux of superoxide, we estimated vascular superoxide in vessel segments treated with DDC, ascorbate, SOD, or EUK-8 as mentioned above. As shown in Figure 6, treatment of aortic segments with 5 mmol/L DDC resulted in a 3.2±0.3-fold increase in the superoxide signal by lucigenin chemiluminescence (P<0.05). This DDC-mediated increase in superoxide was reduced 55%, 81%, and 90% by 0.1, 1, and 10 mmol/L ascorbic acid, respectively (P<0.001 for dose response by ANOVA). In fact, treatment with either 10 mmol/L ascorbic acid, 300 μmol/L EUK-8, or 300 IU/mL SOD reduced the superoxide signal in DDC-treated aortic segments to near control levels (P=NS versus control). Thus, although ascorbic acid, EUK-8, and SOD all reduced vascular superoxide in DDC-treated aortic segments, only EUK-8 and SOD improved EDNO-mediated arterial relaxation.

**Ascorbic Acid and Superoxide Competition for *NO**

The interaction of *NO and superoxide results in the formation of peroxynitrite that spontaneously reacts with tyrosine to form 3-nitrotyrosine. Incubation of tyrosine (2 mmol/L) with an equimolar flux of superoxide (2.3 μmol · L⁻¹ · min⁻¹ from pyrogallol autoxidation) and *NO (2.1 μmol · L⁻¹ · min⁻¹ from DEA-NO) for 15 minutes readily produced 3-nitrotyrosine (2.1±0.31 μmol/L; Figure 7) indicating the formation of peroxynitrite. Both SOD and EUK-8 were able to compete effectively with *NO for superoxide at concentrations exceeding 0.2 μmol/L and 1 μmol/L, respectively (both P<0.05 versus PSS alone by ANOVA; Figure 7). In contrast, ascorbic acid was only partially effective in competing with *NO for superoxide even at a concentration of 10 mmol/L (Figure 7). Thus, ascorbic acid is not as effective as SOD or EUK-8 in competing with *NO for superoxide.

**Discussion**

The data presented here indicate that ascorbic acid does not compete effectively with *NO for superoxide at physiologi-
physiological superoxide flux, ascorbic acid (10 mmol/L), pyrogallol autoxidation (Figure 3), yet only SOD prevented (1 mmol/L) was incubated for 15 minutes with pyrogallol bimolecular rate constant for this reaction is 2.7 to 3.3 ascorbic acid is an effective scavenger of superoxide, the formation of 3-nitrotyrosine was determined by HPLC as described in Materials and Methods. Data are mean±SEM derived from 3 independent experiments. *P<0.05 vs PBS alone by 1-way ANOVA.

Figure 7. Competition with *NO for superoxide. Tyrosine (1 mmol/L) was incubated for 15 minutes with pyrogallol (200 μmol/L) and DEA-NO (20 μmol/L) producing an equimolar flux of superoxide (2.3 μmol·L·min⁻¹) and *NO (2.1 μmol·L·min⁻¹), respectively. Incubations were performed in PBS alone (●) or in PBS containing the indicated concentrations of SOD (▲), EUK-8 (●), or ascorbic acid (■). After incubation, the formation of 3-nitrotyrosine was determined by HPLC as described in Materials and Methods. Data are mean±SEM derived from 3 independent experiments. *P<0.05 vs PBS alone.
ported into cells and along with glutathione is a major determinant of intracellular redox state and antioxidant defenses. Intracellular concentrations of ascorbic acid have been reported in the range of 1.3 to 2.5 mmol/L, and our data (Figures 2 and 7) suggest these concentrations just begin to support effective competition between ascorbic acid and NO for superoxide. Therefore, it is not likely that the improvement in EDNO-mediated arterial relaxation that we previously observed with oral ascorbic acid was purely a consequence of superoxide scavenging by increased intracellular ascorbate.

The source(s) of superoxide in the blood vessel wall remains unclear. In normal vessels, superoxide can be detected throughout the blood vessel wall although the endothelium and adventitia are most notable. In hypercholesterolemia and atherosclerosis, there is evidence for increased activity of xanthine oxidase either within or closely associated with the endothelium. Because NO is freely permeable in biologic tissues, any site of excess superoxide generation will have some impact on EDNO-mediated responses. In contrast, superoxide is not membrane permeable, and our data suggest that competition with NO for superoxide by ascorbic acid will be highly dependent on the site of superoxide generation. For example, impairment of EDNO-mediated arterial relaxation caused by extracellular superoxide generation by xanthine oxidase is not subject to modification by ascorbic acid because the plasma and extracellular ascorbic acid concentration is typically <150 μmol/L. Intracellular source(s) of superoxide that impair EDNO responses, however, may be subject to the action of ascorbic acid by virtue of its higher concentration (1 to 2.5 mmol/L) in the cytosol (Figure 2).

We observed a discrepancy in the action of ascorbic acid that depended on the site of superoxide generation. In vessels treated with DDC to produce an intracellular superoxide flux, ascorbic acid (10 mmol/L) did not improve EDNO-mediated arterial relaxation (Figure 5). In contrast, 10 mmol/L ascorbic acid did improve EDNO responses with an extracellular arterial relaxation (Figure 5). In contrast, 10 mmol/L ascorbic acid did not improve EDNO-mediated arterial relaxation caused by extracellular superoxide dismutase activity. The results depicted in Figure 5 may reflect, in part, some component of NO scavenging. It is unlikely, however, that NO scavenging accounts for much of the response to DDC because treatment with EUK-8 normalizes arterial relaxation to acetylcholine.

In summary, the data presented here indicate that ascorbic acid is not likely to prevent the interaction of NO and superoxide at concentrations that are routinely achieved in plasma or extracellular fluids (<150 μmol/L). These observations are supported by kinetic data indicating that superoxide reacts with NO at a rate that is 10-fold greater than the rate at which superoxide reacts with ascorbic acid. Within the cell cytosol, however, ascorbic acid concentrations (1 to 2.5 mmol/L) begin to approach those needed to support ascorbic acid competition with NO for superoxide. These data indicate that effects of ascorbic acid attributed to preventing the interaction of NO with superoxide must be interpreted with some caution.

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