Ascorbic Acid–Induced Modulation of Venous Tone in Humans

Matthias Grossmann, Dobromir Dobrev, Herbert M. Himmel, Ursula Ravens, Wilhelm Kirch

Abstract—Ascorbic acid appears to have vasodilatory properties, but the underlying mechanisms are not well understood. The aims of this study were to define the acute effects of locally infused ascorbic acid in human veins and to explore underlying mechanisms by using pharmacological tools in vivo. Ascorbic acid was infused in dorsal hand veins submaximally preconstricted with the α₁-adrenoceptor agonist phenylephrine or with prostaglandin F₂α in 23 healthy male nonsmokers, and the venodilator response was measured. Ascorbic acid produced dose-dependent dilation with maximum reversal of constriction of 38±4% in phenylephrine-preconstricted veins and of 51±13% in prostaglandin F₂α-preconstricted veins. Oral pretreatment with the cyclooxygenase inhibitor acetylsalicylic acid or local coinfusion of ascorbic acid and the nitric oxide synthase inhibitor N⁶G-monomethyl-L-arginine had no effect, but coinfusion of ascorbic acid and methylene blue (to inhibit cGMP generation) abolished venodilation. Coinfusion of ascorbic acid and the nonselective potassium channel blocker quinidine abolished venodilation, whereas the inhibitor of ATP-dependent potassium channels glibenclamide had no effect. In cultured bovine endothelial cells, ascorbic acid did not affect intracellular calcium concentration but blunted the response to ATP or digitonin exposure. Ascorbic acid, in millimolar concentrations, dilates human hand veins, presumably by activation of vascular smooth muscle potassium channels through cGMP. This activation is independent of eNOS-mediated nitric oxide synthesis and cyclooxygenase products and does not involve ATP-dependent potassium channels. (Hypertension. 2001;37:949-954.)

Key Words: vitamins  ■  endothelium  ■  vasodilation  ■  nitric oxide  ■  prostaglandins  ■  potassium channels  ■  veins

Ascorbic acid (vitamin C) is the main water-soluble antioxidant in human plasma. It improves endothelium-dependent vasodilation in patients with non–insulin-dependent diabetes, heart failure, and hypertension or in long-term smokers. Although ascorbic acid has been suggested to act by enhancing the effects of liberated endothelial vasodilators, the mechanism of its vascular effects is not well characterized.

Ascorbic acid scavenges reactive oxygen species including superoxide, protects isolated LDL against oxidative modification, and plays an important role in the regulation of intracellular redox state. It prevents nitrate tolerance in coronary arteries in vivo and augments the production of platelet cGMP in vitro. In addition, ascorbic acid maintains coronary vasodilation and production of cGMP in platelets during prolonged infusion of glyceryl trinitrate in patients with congestive heart failure, providing further evidence that it impedes development of nitrate tolerance. In these studies, it was not investigated whether ascorbic acid produces any direct effects in veins despite the fact that these vessels are the primary target for nitrate-induced vasodilation. Moreover, venodilatory effects of ascorbic acid should be of great clinical importance because they are expected to improve the efficacy of nitrates and prevent nitrate tolerance.

Oval doses of ascorbic acid as low as 500 mg per day for 30 days or 6 months slightly lower systolic and mean arterial blood pressure. Because direct vasodilation by ascorbic acid is not present in the brachial artery, this effect could be caused by dilation of the venous vasculature. Venodilation decreases venous blood return to the heart, which determines cardiac output. Simultaneous determinations of cardiac function and venous return curves have shown that maximal reflex change in venous capacitance could alter cardiac output up to 40%. Therefore, the decrease in systolic blood pressure (which mirrors a fall in stroke volume) found in the above-mentioned studies could be attributed to venodilation.

In previous investigations of drug-induced dilation of the preconstricted human hand veins, in which we have used ascorbic acid as a model antioxidant, we observed substantial vasodilation. This effect was present at local concentrations (up to 10 mmol/L) that did not dilate the brachial artery. Here we studied the direct venodilatory effects of ascorbic acid in the same model to elucidate the underlying mechanism of action by using various pharmacological tools. Putative endothelium-dependent effects were investigated by blocking nitric oxide (NO) synthase with N⁶G-monomethyl-L-arginine (L-NMMA) and cyclooxygenase with acetylsalicylic

Received March 24, 2000; first decision May 2, 2000; revision accepted August 28, 2000.
From the Institutes of Clinical Pharmacology (M.G., W.K.) and Pharmacology and Toxicology (D.D., H.M.H., U.R.), Medical Faculty of the University of Technology Dresden (Germany).
Correspondence to Dr med Matthias Grossmann, Institut für Klinische Pharmakologie Bobenheim, Richard-Wagner-Strasse 20, 67269 Grünstadt, FRG.
E-mail grossmann@ikp.de
© 2001 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
Blood Pressure, Heart Rate, Basal Vein Size, Preconstriction Dose, and Extent of Preconstriction

<table>
<thead>
<tr>
<th>Drug Used in Experiment</th>
<th>Before ASC</th>
<th>During ASC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>SBP, mm Hg</td>
</tr>
<tr>
<td>Phenylephrine + ASC</td>
<td>9</td>
<td>131 ± 8</td>
</tr>
<tr>
<td>Phenylephrine + ASA + ASC</td>
<td>5</td>
<td>140 ± 6</td>
</tr>
<tr>
<td>Phenylephrine + L-NMMA + ASC</td>
<td>5</td>
<td>151 ± 6</td>
</tr>
<tr>
<td>Phenylephrine + methylene blue + ASC</td>
<td>6</td>
<td>135 ± 6</td>
</tr>
<tr>
<td>PGF2α + ASC</td>
<td>6</td>
<td>125 ± 6</td>
</tr>
<tr>
<td>PGF2α + glibenclamide + ASC</td>
<td>6</td>
<td>140 ± 6</td>
</tr>
<tr>
<td>PGF2α + quinidine + ASC</td>
<td>6</td>
<td>131 ± 8</td>
</tr>
</tbody>
</table>

Data are mean values ± SEM. ASC indicates ascorbic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure; and HR, heart rate.

Direct Effects of Ascorbic Acid on Dorsal Hand Vein Distensibility

After ~80% preconstriction of the basal vein size (see Table) was obtained with phenylephrine (47 to 1500 ng/min), complete dose-response curves to ascorbic acid (5 to 6000 μg/min) were constructed in 9 subjects. On separate study days, 6 subjects received ascorbic acid after 80% preconstriction of the basal vein size was obtained with PGF2α (a vasoconstrictor independent of adrenergic mechanisms); dose range, 59 to 7500 ng/min).

Five additional study protocols with similar basic design have been performed to explore the underlying mechanism of ascorbic acid–induced venodilation. Three sets of experiments, performed on separate study days, used phenylephrine as preconstrictor. To explore the involvement of cyclooxygenase products in venodilation, oral aspirin (1 g) was given 2 hours before a dose-response curve to ascorbic acid was constructed in 5 subjects. Five subjects received increasing doses of ascorbic acid in the presence of L-NMMA (6.3 μmol/min) on a separate study day to evaluate the contribution of NO. To determine whether generation of cGMP is involved, 6 subjects received increasing doses of ascorbic acid in the presence of methylene blue (13 μg/min=10 μmol/L), a specific guanylyl cyclase inhibitor.

To explore the involvement of potassium channels, 2 other experiments were carried out in PGF2α-preconstricted veins because the nonselective potassium channel inhibitor quinidine is reported to also block α-adrenoceptors. First, 6 subjects received ascorbic acid with and without coinfusion of a constant dose of quinidine gluconate (83 μg/min=50 μmol/L) on separate study days. This dose of quinidine gluconate had no effect on PGF2α-induced preconstriction in preliminary experiments in 2 subjects (data not shown). Second, 6 subjects received ascorbic acid with coinfusion of a constant dose of glibenclamide (20 μg/min=12 μmol/L) during the last 4 doses of ascorbic acid (180 to 6000 μg/min). Glibenclamide inhibits ATP-dependent potassium channels in the human hand vein in vivo.

In Vitro [Ca2+], Measurements in Endothelial Cells

The cytосolic calcium concentration ([Ca2+]i) in cultured bovine aortic endothelial cells (BAEC) was quantified by spectrofluorimetry with the Ca2+-sensitive fluorescent dye fura-2/AM, according to previously published methods.

Data Analysis

Individual dose-response curves to ascorbic acid could not be adequately fitted by an Emax model because no maximum effect was established. Therefore, responses to drug treatments were compared with the maximum ascorbic acid dose used (6000 μg/min). All results are expressed as mean ± SEM unless otherwise stated. Results were analyzed by paired or unpaired t test or ANOVA and Student-Newman-Keuls test. A value of P<0.05 was considered statistically significant.
Results

Local infusion of ascorbic acid into the dorsal hand veins of healthy volunteers, with or without concomitant local or systemic drugs, had no local or systemic adverse effects and did not influence blood pressure or heart rate (see Table).

Direct Effects of Ascorbic Acid on Dorsal Hand Vein Distensibility

Infusion of ascorbic acid into phenylephrine-preconstricted hand veins caused vasodilation in a dose-dependent manner (Figure 1). The venodilatory response reached a maximum of 38±4% at an infusion rate of 6000 μg/min. In veins preconstricted with PGF₂α, ascorbic acid also caused dose-dependent venodilation (maximum 51±13%; Figure 1). The difference between the two curves in Figure 1 did not reach the level of statistical significance.

The venodilation by ascorbic acid reversed rapidly on cessation of the infusion. Venous diameter returned to its phenylephrine-preconstricted or PGF₂α-preconstricted baseline 5 to 10 minutes after ascorbic acid infusion was terminated (data not shown).

Involvement of Endothelium-Dependent Factors

Oral pretreatment with ASA (to inhibit cyclooxygenase) or coinfusion of ascorbic acid and L-NMMA (to inhibit NO synthesis) did not significantly affect ascorbic acid–induced venodilation (Figure 2). To investigate whether ascorbic acid is able to enhance [Ca²⁺]ᵢ in endothelial cells, which could activate the NO synthase and cyclooxygenase, we exposed BAECs to concentrations of ascorbic acid equivalent to the hand vein perfusate that provoked vasodilation in vivo (540, 1600, and 6000 μg/min). Assuming local flow in the hand vein of ~3 mL/min, these equate with concentrations of ascorbic acid of ~1, 3, and 10 mmol/L, respectively. The fluorescence ratio as a measure for [Ca²⁺]ᵢ in BAECs was minimally affected by ascorbic acid up to 3 mmol/L and decreased significantly with 10 mmol/L. (Figure 3, A and B). However, the subsequent addition of ATP and the detergent digitonin increased the fluorescence ratio significantly less than in cells without prior exposure to ascorbic acid, suggesting that ascorbic acid may chelate [Ca²⁺]ᵢ. To test this, the plasma membrane was permeabilized with digitonin, allowing intracellular Fura-2 to saturate with extracellular Ca²⁺.

Involvement of Endothelium-Independent Mechanisms

Figure 4 illustrates the response to ascorbic acid (infusion rate of 6000 μg/min) in phenylephrine-preconstricted veins during treatment with methylene blue (to inhibit generation of ([Ca²⁺]ᵢ)). Under these conditions, cumulative increase of the ascorbic acid concentration attenuates the fluorescence ratio curve (Figure 3C). The inhibition curve was sensitive to variations of [Ca²⁺]ᵢ, and shifted to the right with high [Ca²⁺]ᵢ, (data not shown).

Figure 1. Dose-response curves for ascorbic acid in human dorsal hand veins preconstricted with phenylephrine (●, n=9) and PGF₂α (○, n=6).

Figure 2. Endothelium-dependent effects of vitamin C. In hand veins preconstricted with phenylephrine, ascorbic acid (6000 μg/min) was infused alone (control 1; n=9), after pretreatment with ASA (1 g orally; n=5), and in combination with L-NMMA (6.3 μmol/min; n=5).

Figure 3. Effect of ascorbic acid on [Ca²⁺]ᵢ in endothelial cells in vitro. A, Superimposed representative tracings of fluorescence ratio vs time: a, cumulative addition of ascorbic acid (1, 3, and 10 mmol/L) with subsequent addition of ATP (100 μmol/L), digitonin, and EDTA; b, exposure to ATP (100 μmol/L) alone followed by digitonin and EDTA; arrows indicate addition of compounds. B, Average effects on fluorescence ratio as a measure for [Ca²⁺]ᵢ. Mean values±SEM of 6 experiments with ascorbic acid (solid bars) and of 8 experiments with ATP alone (crisscross bars); asterisks denote significant differences vs control (P<0.05). C, Representative tracing of fluorescence ratio vs time during sequential and cumulative addition of digitonin, ascorbic acid (1, 2, 3, 5, and 10 mmol/L), and EDTA, as indicated by arrowheads; [Ca²⁺]ᵢ in cuvette was 1.8 mmol/L.
cGMP). Methylene blue abolished the venodilation (5±3%; Figure 4).

In PGF₂α-preconstricted veins, coinfusion of the nonelective potassium channel inhibitor quinidine abolished the ascorbic acid–induced venodilation (7±12%), whereas application of glibenclamide (to inhibit ATP-dependent potassium channels) had no effect (Figure 4).

Discussion

To the best of our knowledge, this is the first report on direct effects of ascorbic acid on contractile status in human veins. The ascorbic acid–induced venodilation was affected neither by blockade of NO synthesis with L-NMMA nor by inhibition of cyclooxygenase with ASA. It does not increase [Ca²⁺], ascorbic acid has been shown to stimulate NO production in generation and release of NO and prostacyclin, leading to vasodilation. In our endothelial cell model, millimolar concentrations of ascorbic acid had no effect on basal [Ca²⁺], but blunted the rise of Ca²⁺ that normally follows ATP or digitonin exposure. Therefore, [Ca²⁺]-induced generation of NO and prostaglandins is unlikely to contribute to the venodilatory effect of ascorbic acid.

Because the ATP-induced [Ca²⁺], transient consists of both Ca²⁺ release from intracellular stores and Ca²⁺ influx from the extracellular space and because both ATP-induced and digitonin-induced [Ca²⁺], transients are depressed, the observed attenuation of fluorescence ratio could be caused by chelation of extracellular Ca²⁺. In vivo, chelation of extracellular Ca²⁺ should decrease Ca²⁺ influx into vascular smooth muscle cells, resulting in vasodilatation. Alternative mechanisms of action of ascorbic acid relate to its reducing and scavenger properties. As a reducing agent, ascorbic acid may alter the redox state of soluble guanylyl cyclase in vascular smooth muscle cells, which is sensitized to NO, thus mediating relaxation. Superoxide reduces relaxation by partially inactivating endothelial NO. Because ascorbic acid in millimolar concentration scavenges superoxide radicals, it increases the availability of NO.

Ascorbic acid has been shown to stimulate NO production after 24 hours of incubation with low micromolar concentrations (100 to 200 μmol/L). Because sodium-dependent, carrier-mediated active transport into endothelial cells exists, extended incubation may increase the intracellular concentration of ascorbic acid. Under physiological conditions, this concentration is in the range of 1 to 2.5 mmol/L, that is, ~20 times higher than in plasma.

In our study, blockade of NO synthase with L-NMMA had no effect on ascorbic acid–induced venodilation. However, nonenzymatic NO production has been demonstrated in humans. We cannot exclude that under physiological conditions, nonenzymatic NO production from nitrite occurs, but we assume that the role of nonenzymatic NO production should be negligible because of the nonphysiological acid conditions (threshold=pH 6) required for this reaction. Such pH conditions were unlikely to be present in our study (see Methods).

Although ascorbic acid also increases prostacyclin production in endothelial cells, inhibition of cyclooxygenase with ASA did not modulate ascorbic acid–induced venodilation. Thus, NO and vasodilatory cyclooxygenase products are not involved. It is possible, however, that the time of exposure to ascorbic acid was too short, so that no effect on NO synthase or cyclooxygenase could occur. On the other hand, these
results may indicate a possible endothelium-independent effect of ascorbic acid in the hand vein.

**Involvement of Endothelium-Independent Factors in Ascorbic Acid–Induced Dilation**

Ascorbic acid causes hyperpolarization in various cells, which can originate from activation of potassium channels or of membrane Na⁺-K⁺ ATPase. In our study, quinidine, a nonselective potassium channel inhibitor, completely blocked the ascorbic acid–induced venodilation, suggesting possible involvement of potassium channels. Activation of potassium channels has been described as a key mechanism of smooth muscle relaxation caused by NO and prostacyclin. Hence, ascorbic acid–induced activation of potassium channels in vascular smooth muscle appears to be independent of endothelial vasoactive mediators.

Quinidine is a nonselective inhibitor of potassium channels in the human vasculature. In addition to its ability to block potassium channels, this drug exerts also antidiuretic, anticholinergic, and calcium channel–blocking properties. Interaction with α-adrenoceptors can be excluded because we used PGF₂α as venoconstrictor. Because neither nifedipine nor lidocain dilate human hand veins in vivo, L-type calcium channels or sodium channel–blocking properties are unlikely to contribute. On the other hand, application of high concentrations of atropine significantly dilate phenylephrine-preconstricted human veins (M. Grossmann and D. Dobrev, 1999, unpublished observations), implying involvement of muscarinic receptors. In dose-finding studies for quinidine, we initially applied 500 μg/min, as suggested by Smits et al, and found substantial venodilation in which all the multiple effects of quinidine, including anticholinergic action, could be involved. However, at the dose eventually selected, quinidine had no dilatory effect during 70 minutes of infusion (data not shown). Therefore, quinidine-induced block of ascorbic acid–induced venodilation is likely to be due to nonselective inhibition of potassium channels.

Which potassium channels might be involved? Quinidine modulates voltage-gated (Kᵥ), calcium-activated (KᵥCa), inward rectifier (Kᵢ), and ATP-dependent (KᵦTP) potassium channels. In an attempt to define the type of potassium channels involved, we blocked KᵦTP channels with glibenclamide and found no effect, excluding involvement of KᵦTP channels. Unfortunately, additional experiments with charybdotoxin (selective blocker of KᵥCa) or 4-aminopyridine (selective blocker of Kᵢ) could not be conducted because these compounds cannot be used in humans. Therefore, the type of potassium channel involved in the ascorbic acid–induced venodilation remains elusive.

Activity of potassium channels in smooth muscle cells may be modulated by cGMP. Our finding that methylene blue, an inhibitor of soluble guanylate cyclase, abolished the venodilation indicates a possible involvement of cGMP generation. NO is the major endogenous activator of the guanylyl cyclase in smooth muscle cells. Because in our study an NO-mediated action of ascorbic acid is unlikely, a direct action of ascorbic acid on cGMP generation may be postulated. As previously described, the reducing agent ascorbic acid may activate the cGMP production. Indeed, ascorbic acid relaxes rat and guinea pig thoracic aorta in vitro in a concentration-dependent manner, and this effect is also inhibited by methylene blue.

**Limitations of the Study**

First, the study has been conducted in healthy subjects as a prelude to experiments in patients with coronary artery disease. However, these effects may be missing in this patient population. Second, neither intracellular generation of cGMP nor activation of potassium channels were measured because examination of the in vivo effects of ascorbic acid on production of cGMP and on activation of potassium channels in vascular smooth muscle cells is feasible only in biopsies. Third, we did not investigate the effects of other antioxidants such as vitamin E and β-carotene. Thus, the potential beneficial effects of ascorbic acid are not necessarily representative for other antioxidants.

**Conclusions**

Local infusion of ascorbic acid into preconstricted dorsal hand veins of healthy subjects causes dose-dependent vasodilation that can neither be attributed to stimulation of NO synthase or cyclooxygenase activities nor to increased endothelial [Ca²⁺]. Instead, cGMP formation and potassium channel activation appear to be involved in the response. As a possible explanation for the observed effects, we suggest that ascorbic acid modulates the redox state of soluble guanylyl cyclase, thereby activating cGMP-dependent potassium channels that hyperpolarize the smooth muscle cell membrane and thus induce vasodilation. Thus, further detailed investigations of the direct effects of ascorbic acid on human venous smooth muscle cells are needed because in a clinical setting, venodilatory effects of ascorbic acid are relevant with respect to the wide use of organic nitrates.

**Acknowledgments**

This study was part of the thesis of Birgit Horn, and the authors appreciate her help with the study subjects. The authors also wish to thank Dr Michael J. Jamieson for his critical reading of the manuscript and Trautlinde Grossmann for skillful technical assistance.

**References**


Ascorbic Acid–Induced Modulation of Venous Tone in Humans
Matthias Grossmann, Dobromir Dobrev, Herbert M. Himmel, Ursula Ravens and Wilhelm Kirch

Hypertension. 2001;37:949-954
doi: 10.1161/01.HYP.37.3.949
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/37/3/949

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/