Influence of Vitamin C on Baroreflex Sensitivity in Chronic Heart Failure

Gianfranco Piccirillo, Marialuce Nocco, Antonio Moisè, Marco Lionetti, Camilla Naso, Silvia di Carlo, Vincenzo Marigliano

Abstract—Chronic heart failure (CHF) reduces baroreflex sensitivity. Low baroreflex sensitivity, a risk factor for sudden death, could arise partly from CHF-dependent endothelial dysfunction. Vitamin C at high doses has a protective role against CHF-related endothelial damage. This study was conducted to investigate the effect of vitamin C on baroreflex sensitivity in CHF. A study group of 33 subjects with CHF secondary to postischemic dilated cardiomyopathy with an ejection fraction ≤35% and a control group (11 subjects) underwent assessment of baroreflex sensitivity by the phenylephrine method and an autonomic nervous system study by power spectral analysis. Variables were assessed after infusion of placebo and high doses of vitamin C (2.5 mg). In subjects with CHF, baroreflex sensitivity was significantly higher after vitamin C than after placebo infusion (placebo: 4.1 ± 0.4 versus vitamin C: 5.3 ± 0.5 ms/mm Hg, P < 0.001). Low-frequency of R-R (LFRR), expressed in normalized units (NU) (P < 0.05); LF/high-frequency (HF) ratio (P < 0.05), and LF of SBP (LFsbp) decreased significantly; HF power (P < 0.05), and α-HF (P < 0.001) increased. Conversely, in the control group, baroreflex sensitivity and other spectral variables measured at baseline, after placebo, and after vitamin C infusion remained statistically unchanged (placebo: 10.2 ± 0.1 versus vitamin C: 10.0 ± 0.2 ms/mm Hg, NS). Acute administration of vitamin C at high doses improves baroreflex sensitivity and vagal sinus modulation in patients with CHF. This finding could have notable clinical and therapeutic implications. Key issues to understand are whether the beneficial effect persists during chronic administration and whether it helps to improve survival. (Hypertension. 2003; 41:1240-1245.)

Key Words: autonomic nervous system | baroreflex | vitamins | heart failure | endothelium | vasoconstriction

Endothelial dysfunction associated with heart failure provokes exercise intolerance and is implicated in the progression of disease. Evidence from animal studies suggests that diminished baroreflex sensitivity is caused by endothelial dysfunction. In particular, in experimentally induced endothelial dysfunction, the decreased prostacyclin and increased thromboxane concentrations are associated with reduced baroreflex impulses from the carotid artery. This observation may in part explain why many clinical conditions involving endothelial dysfunction, including chronic heart failure (CHF), arterial hypertension, aging, and post–myocardial infarction attenuate baroreflex function. Although low baroreflex sensitivity in CHF unquestionably arises mainly from increased sympathetic activity, an appreciable amount could also reflect endothelial dysfunction. Reduced baroreflex sensitivity in CHF may therefore be causally related to endothelial dysfunction.

Recent evidence shows that low baroreflex sensitivity, measured by the phenylephrine method, is a predictor of sudden death from malignant ventricular arrhythmias. Hence, it underscores the potential usefulness of drugs that could improve baroreflex sensitivity and autonomic nervous system function in patients with CHF, thus lowering mortality rates from sudden death.

The mechanism through which vitamin C improves endothelial dysfunction in CHF remains unclear. Some investigators consider it unlikely that vitamin C reduces oxidative stress, favoring inhibition of endothelial cell apoptosis. Regardless of how it acts, by improving endothelial cell function, vitamin C could improve autonomic nervous system control through 2 mechanisms: indirectly, by improving hemodynamics, or directly, by acting on subendothelial nerve endings at the baroreceptor level (carotid, aortic, or cardiopulmonary). Possible support for a direct action comes from the recent observation that endothelium-derived nitric oxide increases vagal activity in patients with CHF and that acute infusion of vitamin C influences nitric oxide–mediated vasodilation. Acute administration of vitamin C might therefore improve local nitric oxide production, thus directly stimulating the vagus nerve.

Prompted by these findings, we designed this study to investigate whether high-dose vitamin C improves baroreflex sensitivity, autonomic nervous control, and hemodynamic variables in patients with CHF. We assessed baroreflex...
sensitivity with the phenylephrine method and cardiovascular autonomic control by power spectral analysis, and we also measured changes in brachial artery flow after vitamin C infusion.\textsuperscript{18,21}

**Methods**

For this study, we selected 33 outpatients (29 men and 4 women) who had stable CHF secondary to ischemic dilated cardiomyopathy and 11 healthy control subjects (9 men and 2 women) (Table 1). Patients with CHF were taking standard medications for heart failure, including enalapril or losartan, furosemide, spironolactone, carvedilol, digoxin, and acetylsalicylic acid. Of the 33 patients with CHF studied, 11 belonged to New York Heart Association class II and 22 to class III.

After a 30-minute rest lying down, the subject underwent a first assessment of baroreflex sensitivity (baseline). Vitamin C or placebo was then randomly administered in 2 separate sessions. During the drug session, 2.5 mg of vitamin C was infused over 5 minutes;\textsuperscript{16,17} during the placebo session, participants received infusion of saline alone. Fifteen minutes after the infusions ended, baroreflex sensitivity was measured by the phenylephrine method.

During the baroreflex study, healthy control subjects and those with CHF had to breathe at 20 breaths per minute in time with a metronome, and each subject underwent a simultaneous recording from a single ECG lead for beat-to-beat blood pressure and respiratory rate.

Short segments (256 beats) recorded under respiratory control before the 2 phenylephrine studies were used to determine R-R intervals, systolic blood pressure (SBP), stroke volume, cardiac output, peripheral resistance, and spectral data.\textsuperscript{22,23} Data were acquired during controlled respiration to prevent abnormal respiratory patterns typical of CHF from interfering with the results of autonomic assessment.\textsuperscript{24,25}

Baroreflex sensitivity was determined with the method originally proposed by Smyth et al\textsuperscript{26} and used by Mortara and colleagues.\textsuperscript{14}

Stationary 256-beat segments of ECG, blood pressure, and respiratory recordings were analyzed with an autoregressive algorithm.\textsuperscript{10,27} We then determined the total power of R-R intervals (R-R) and SBP and the total spectral density of these variables. For R-R and SBP, we calculated the following spectral components: a high-frequency (HF) component (0.03 Hz Eq.), a low-frequency (LF) component (0.07 Hz Eq.), and a very-low frequency component (<0.03 Hz Eq.).\textsuperscript{9,12,27} The resulting spectral data were transformed into the natural logarithm of the variable (ln). The relative value of each spectral power component was also measured and expressed in normalized units (NU).\textsuperscript{9,12,27} We calculated spontaneous baroreceptor sensitivity with alpha (α) indexes.\textsuperscript{9,11,12,29,30} From the same 256-beat segment, we then calculated a time-domain index: the root mean square of successive R-R intervals differences (RMSSD), an index that provides another marker of sinus vagal activity.\textsuperscript{27}

In all subjects with CHF, we also measured forearm blood flow–mediated dilation after infusion of vitamin C or placebo.\textsuperscript{17} Flow-mediated dilation data were expressed as percentage changes in the baseline brachial artery diameter.

One-way ANOVA was used to compare the general characteristics, baroreflex sensitivity, and other data in the CHF subjects and control group. Repeated-measures ANOVA was used to evaluate the differences between placebo and vitamin C infusion. A probability value of <0.05 was considered to indicate statistical significance.

**Results**

No differences were found between the 2 groups for age, arterial pressures, or body mass index (Table 1). Heart rate, ejection fractions, and noninvasive hemodynamic variables differed significantly between subjects with CHF and the control group (Table 1). In subjects with CHF, administration of vitamin C induced no significant changes in stroke volume (from 13.6 ± 1.0 to 14.3 ± 1.1 mL/m\(^2\), NS), cardiac output (from 0.95 ± 0.04 to 0.97 ± 0.06 L · min\(^{-1}\) · m\(^{-2}\), NS), and peripheral resistance (from 2437 ± 123 to 2389 ± 151 dyne · s · cm\(^{-5}\) · m\(^2\), NS). Similarly, in control subjects, it also left these variables practically unchanged (stroke volume: from 22.8 ± 1.1 to 22.3 ± 1.0 mL/m\(^2\), NS; cardiac output: from 1.58 ± 0.10 to 1.49 ± 0.20 L/min per m\(^2\), NS; and peripheral resistance from 1351 ± 43 to 1299 ± 58 dyne · s · cm\(^{-5}\) · m\(^2\), NS). In subjects with CHF, vitamin C infusion slightly increased the R-R interval (P < 0.05) (Table 2).

Baseline baroreflex sensitivity values were lower in patients with CHF than in normal subjects (4.1 ± 0.5 versus 10.0 ± 1.0 ms/mm Hg, P < 0.001). In subjects with CHF, no difference was found between baseline baroreflex sensitivity and values measured after placebo, but baroreflex sensitivity values were significantly higher after vitamin C than after placebo (from 4.1 ± 0.4 to 5.3 ± 0.5 ms/mm Hg, P < 0.001) (Figures 1 through 3). Expressed in relative terms, the mean percentage increase in baroreflex sensitivity was 22.6 ± 3.9%. Of the 33 subjects with CHF studied, 21 (64%) responded to vitamin C infusion; no significant difference was found for the other study variables in responders and nonresponders. Conversely, in the control group, baroreflex sensitivity remained unchanged at baseline, after placebo and after vitamin C (placebo: 10.2 ± 0.1 versus vitamin C: 10.0 ± 0.2 ms/mm Hg, NS). In subjects with CHF, to obtain good baroreflex sensitivity responses required significantly higher phenylephrine doses in the 3 experimental conditions (baseline, pla-

**TABLE 1. Baseline Characteristics of Study Subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CHF Subjects (n = 33)</th>
<th>Control Subjects (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56 ± 1</td>
<td>55 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>26 ± 0.5</td>
<td>26 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>75 ± 1</td>
<td>70 ± 1</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122 ± 2</td>
<td>116 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>70 ± 2</td>
<td>70 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>25 ± 0.7</td>
<td>59 ± 0.9</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Stroke volume index, mL/m(^2)</td>
<td>14 ± 1</td>
<td>23 ± 1</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cardiac index, L · min(^{-1}) · m(^{-2})</td>
<td>0.95 ± 0.04</td>
<td>1.58 ± 0.1</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Vascular resistance, dyne · s · cm(^{-5}) · m(^2)</td>
<td>2437 ± 123</td>
<td>1351 ± 43</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. CHF indicates chronic heart failure.
cebo, and vitamin C) (baseline: 3.1±0.2 μg/kg; placebo: 2.9±0.2 μg/kg; vitamin C: 3.7±0.1, P<0.05, P<0.05).
These doses yielded the same pressure increases (baseline: from 123±5 to 144±2 mm Hg; placebo: from 120±5 to 140±2 mm Hg; vitamin C: from 119±5 to 141±2 mm Hg). Conversely, in the control group, among the various study conditions (baseline, placebo, and vitamin C), no significant differences were found either in phenylephrine levels or in the arterial pressures reached during assessment of baroreflex sensitivity.

In subjects with CHF, vitamin C infusion induced a significant change in LFnu NU (P<0.001), HFnu power (P<0.001), HFnu NU (P<0.001), LF/HF (P<0.05), LFnuBP (P<0.05), and αHF (P<0.001) (Table 2 and Figure 3). Conversely, in control subjects it left all these spectral variables significantly unchanged (Table 2). The RMSSD increased significantly after vitamin C infusion but did so only in subjects with CHF (P<0.001) (Table 2).

Baroreflex sensitivity values (P<0.05) and ejection fractions were higher (P<0.05) and baseline heart rates were lower (P<0.05) in subjects who were taking carvedilol than in those who were not, but in both groups vitamin C infusion led to similar changes in baroreflex sensitivity. Similarly, no significant difference was found between the increase in baroreflex sensitivity according to the use or nonuse of digoxin. Subgrouping subjects with CHF as responders and nonresponders to vitamin C disclosed no significant differences in autonomic nervous system or hemodynamic variables between the 2 groups.

Flow-mediated dilation increased after intravenous vitamin C infusion (from 4.1±0.9% to 8.0±0.8%, P<0.05). Improvements in flow-mediated dilation did not correlate with power spectral data or baroreflex sensitivity.

The mean coefficient of variation for the 2 different baroreflex assessments was 0.2±0.01 ms/mm Hg (3.8±0.5%).

Discussion
The major point of this work was that the administration of vitamin C improved baroreflex sensitivity and vagal cardiac control in subjects with CHF.
This is a clinical syndrome that profoundly upsets autonomic nervous system control of the cardiovascular system and endothelial function. Baroreflex sensitivity measured by the phenylephrine method expresses the ability to reduce the heart rate in function of an increase in arterial pressures. This ability is closely linked to vagal nerve functionality. The vagus nerve exerts an antiarrhythmic function, thus protecting against sudden death.31 Because vagal nerve function is strongly compromised in CHF, sympathetic activity predominates. In this study, we showed that baroreflex sensitivity improves after administration of vitamin C. However, in our subjects, no hemodynamic benefits accompanied the improved baroreflex function, since acute administration of vitamin C left arterial pressures, stroke volume, cardiac output, and peripheral resistances unchanged. The only change was a slight increase in the mean R-R intervals. Hence, the changed baroreflex sensitivity that we observed arises from the increased cardiac vagal response to the pressure stimulus. In other words, vitamin C increases the ability of the autonomic nervous system, especially the vagus nerve, to respond to pressure variations. Our spectral data confirmed this behavior by disclosing an absolute and relative (in normalized units) increase of HF and α-HF, thus indicating augmented sinus node vagal modulation and increased baroreflex sensitivity.9,11,12,29 The increased RMSSD was in line with these findings. Our spectral data also seemed to indicate diminished sympathetic sinus modulation. Accordingly, we observed a relative reduction (normalized units) of LF and LF/HF.10,27 In some studies showing that vitamin C increases arterial blood flow in patients with CHF in response to a hyperemic reaction, the investigators attributed the increased flow to improved endothelial function.17 The vitamin C-induced increase in arterial blood flow they observed, however, was a response to a hyperemic reaction (vasodilation) produced through transient ischemia. In patients with CHF, we repeated the same maneuver to seek a possible relation between endothelium-dependent vasodilation and autonomic indexes. Because we found no association between variations in flow-mediated dilation and autonomic changes, the 2 phenomena are presumably not interdependent. Our data therefore seem to indicate a direct effect of vitamin C on vagal activity. Even so, like others, we also found that vitamin C infusion had a slight vasodilatory action. During baroreflex sensitivity assessment, we therefore had to use higher phenylephrine doses in subjects with CHF than in...
healthy control subjects. A point to remember is that as an α-agonist, phenylephrine has a vasoconstricting action. The higher phenylephrine doses needed after infusion of vitamin C indicate changes drug sensitivity, possibly caused by a vitamin C–mediated increase in cardiovagal baroreflex buffering.32

Although our study shows that vitamin C infusions induce an acute increase in vagal activity, it gives no information on the possible effects during chronic administration. If baroreflex sensitivity increases to a similar extent after chronic administration of vitamin C, then these findings would be of extreme clinical interest. Improved baroreflex sensitivity along with a prolonged increase in vagal activity might ultimately improve the hemodynamic status by reducing heart rate and myocardial oxygen demand. By strengthening the vagal antiarrhythmic effect, it might also reduce the risk of sudden death. Though these events remain purely conjectural, they call for further studies planned to assess the possible cardiac benefits of long-term therapy with vitamin C.

Some investigators maintain that endothelial dysfunction diminishes baroreflex sensitivity. Accordingly, experimental evidence shows that a reduction in prostacyclin, an increase in free oxygen radicals, and the reduced endothelium-dependent synthesis of nitric oxide associated with endothelial dysfunction are all factors that reduce baroreflex sensitivity.7,19,20 Vitamin C, a potent antioxidant, improves endothelial function. Besides reducing oxidative stress,17 it also acts to suppress the induction of endothelial cell apoptosis by cytokines, tumor necrosis factor-α, and angiotensin II.18 Whatever the underlying mechanism, our study provides evidence that vitamin C has the ability to increase baroreflex sensitivity and does so independent of hemodynamic improvement. Our findings on endothelial involvement in autonomic control in CHF receive further support from the observation that carvedilol, the only β-blocker that has antioxidant effects,33 is also the β-blocker that gives the best results in CHF.34 Although an endothelium involvement in the improved baroreflex sensitivity seems the most likely hypothesis, it is not the only one. The data from our study leave open the possibility that vitamin C acts directly on the sinoatrial node or on the autonomic respiratory pattern.35

Last, we underline that low baroreflex sensitivity as determined with the phenylephrine method is associated with high mortality rates in CHF8,15 and in patients with post–myocardial infarction.13 Despite the various approaches tried, few data have emerged as a guide to specific therapy. For example, although β-blockers reduce sympathetic activity and increase survival in CHF and post–myocardial infarction, they have scarce direct influence on vagal activity; digitalis increases vagal activity but leaves survival in CHF unchanged; and transdermal scopolamine (a vagal antagonist at small doses) increases vagal activity,36 but how it affects survival in CHF remains unknown. The results of this study should provide an impetus for planning trials designed not to investigate an alternative to existing drugs but to investigate a new therapy that has the ability to augment cardiac vagal activity. These preliminary data need further clinical and pathophysiological confirmation.

Perspectives
In conclusion, our data indicate a direct effect of vitamin C on autonomic control in subjects with CHF. This finding, if confirmed also during chronic administration of vitamin C, is useful because it could permit sympathovagal control to be restored to a level sufficient for reducing the onset of malignant ventricular arrhythmias. The mechanism underlying the improved autonomic control is probably linked to a direct antioxidant effect of vitamin C on CHF-dependent endothelial damage, though this hypothesis remains to be proved. The next step is a study designed to assess endothelium–produced nitric oxide in relation to changes in spectral and nonspectral autonomic indexes during acute and chronic administration of vitamin C.

References


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