Carvedilol Inhibits Reactive Oxygen Species Generation by Leukocytes and Oxidative Damage to Amino Acids

Paresh Dandona, MD; Rajaram Karne, MBBS; Husam Ghanim, BS; Wael Hamouda, MS; Ahmad Aljada, PhD; Cesar H. Magsino, Jr, MD

Background—The purpose of this study was to test whether carvedilol has an antioxidant effect in humans in vivo.

Methods and Results—We administered 3.125 mg of carvedilol twice daily to normal subjects for 1 week. ROS generation by polymorphonuclear leukocytes and mononuclear cells fell from 314±183.43 and 303±116 mV to 185±157 and 189±63 mV (P<0.025), respectively. m-Tyrosine fell from 4.24±0.99 to 4.03±0.97 ng/mL (P=0.01), and o-tyrosine fell from 4.59±1.10 to 4.24±0.99 ng/mL (P=0.004) in the absence of a change in phenylalanine concentrations.

Conclusions—We conclude that carvedilol significantly inhibits ROS generation by leukocytes and oxidative conversion of phenylalanine to m- and o-tyrosine. (Circulation. 2000;101:122-124.)

Key Words: carvedilol ■ oxygen ■ leukocytes ■ amino acids

It has been suggested that carvedilol may provide greater benefit than traditional β-blockers in chronic heart failure because of its antioxidant actions that synergize with its nonspecific β- and α-blocking effects.1 Carvedilol has been shown to inhibit lipid peroxidation of myocardial cell membranes and thus protect endothelial, neuronal, and vascular smooth muscle cells from oxygen radical–mediated injury.2 Carvedilol has also been shown to scavenge peroxyn and hypochlorous radicals in chemical systems in vitro.3 In the only study in humans, carvedilol was shown to have antioxidant actions in patients treated with moderate doses of 25 mg/d as assessed by suppression of ex vivo LDL oxidation and reduction of anti–oxidized LDL antibodies in vivo.4

Ortho-tyrosine (o-tyrosine) and meta-tyrosine (m-tyrosine) have recently been shown to be useful markers of oxidative damage to phenylalanine, because they are formed after reactive oxygen species (ROS) attack on phenylalanine. Thus, their concentration is considered to be an index of oxidative damage to amino acids and proteins.

We undertook this study to investigate the effect of carvedilol administration on ROS generation by polymorphonuclear leukocytes (PMNLs) and mononuclear cells (MNCs). We also measured o-tyrosine and m-tyrosine in plasma as indices of oxidative damage to phenylalanine.

Methods

Eight normal subjects 26 to 33 years old voluntered for the study. The study was approved by the Institutional Review Board of the State University of New York at Buffalo. Written, informed consent was obtained from each subject. None of the subjects were on any medications, including NSAIDS, vitamin E, or other antioxidants.

Fasting blood samples were collected at baseline in tubes with EDTA as an anticoagulant. The subjects were given 3.125 mg of carvedilol PO twice a day for 7 days. On day 8, another fasting blood sample was collected as above. ROS generation by PMNLs and MNCs and levels of o-tyrosine and m-tyrosine in plasma were measured at baseline and on day 8.

A group of 6 control subjects, not given any drugs, also had 2 fasting blood samples taken 1 week apart without any drug intervention.

Preparation of PMNLs and MNCs

PMNLs and MNCs were prepared, washed, and suspended in HBSS as previously described.5

Measurement of ROS Generation

Respiratory burst activity of PMNLs and MNCs was measured by detection of superoxide radical via chemiluminescence.6 Five hundred microliters of PMNLs or MNCs (2×10⁶ cells) was delivered into a Lumigaggrometer (Chronolog) plastic flat-bottom cuvette to which a spin bar was added. Fifteen microliters of 10 mmol/L luminol was then added, followed by 1 μL of 10 mmol/L formylmethionylleucinylphenylalanine (FMLP). Chemiluminescence was recorded for 15 minutes (a protracted record after 15 minutes did not alter the relative amounts of chemiluminescence produced by various cell samples). Our method, developed independently, is similar to that published by Tosi and Hamedani.7 The interassay coefficient of variation (CV) for this assay is 6%. We have further established that in our assay system, there is a dose-dependent inhibition of chemiluminescence by superoxide dismutase and catalase: superoxide dismutase inhibited chemiluminescence by 82% at 10 μg/mL, whereas catalase inhibited chemiluminescence by 47% at 40 μg/mL. Chemiluminescence is also inhibited by dihydrolipoamide chloro (data not shown), a specific inhibitor of NADPH oxidase, the enzyme responsible for the production of superoxide radicals.8 Our assay system is exquisitely sensitive to dihydrolipoamide chloride at nanomolar concentrations.

Reference


Assay of \(\text{o-Tyrosine, m-Tyrosine, and Phenylalanine}\)

\(\text{o-Tyrosine, m-tyrosine, and phenylalanine were measured by high performance liquid chromatography using the technique described by Ishimitsu et al.}\,^9\]

Statistical Analysis

Comparisons of the ROS generation values at baseline and on day 8 were carried out by Wilcoxon rank sum test, because the distribution of the values was not normally distributed. The values of \(\text{o-tyrosine}\) and \(\text{m-tyrosine}\) before and after carvedilol were compared by paired \(t\) test.

Results

ROS generation by PMNLs at baseline was \(313.75 \pm 183.43\) mV (mean \(\pm\) SD). After carvedilol administration, ROS generation fell to \(185.00 \pm 156.98\) mV. The mean fall was \(43.75 \pm 15.31\%\) (range, 20\% to 65\%; \(P = 0.025\)) (Figure 1). The mean ROS generation by PMNLs in the control group was \(544 \pm 85\) mV at baseline, and it was \(515 \pm 80\) mV 1 week later (\(P = \text{NS}\)).

ROS generation by MNCs at baseline was \(302.50 \pm 115.70\) mV (mean \(\pm\) SD). After carvedilol administration, ROS generation fell to \(189.25 \pm 63.09\) mV. The mean fall was \(34.77 \pm 14.58\%\) (range, 21\% to 57\%; \(P = 0.025\)) (Figure 2). Mean ROS generation by MNCs in control subjects was \(336 \pm 45\) mV at baseline and \(330 \pm 42\) mV a week later (\(P = \text{NS}\)).

Plasma phenylalanine concentration did not change after carvedilol. Plasma \(m\)-tyrosine concentration fell from \(4.24 \pm 0.99\) to \(4.03 \pm 0.97\) ng/mL (\(P = 0.01\)). The ratio of \(m\)-tyrosine to phenylalanine changed from \(0.35 \pm 0.07\) to \(0.33 \pm 0.07\) mmol/mol phenylalanine (\(P = 0.005\)).

Plasma \(o\)-tyrosine concentration fell from \(4.59 \pm 1.10\) to \(4.24 \pm 0.90\) ng/mL (\(P = 0.004\)). The ratio of \(o\)-tyrosine to phenylalanine changed from \(0.35 \pm 0.07\) to \(0.33 \pm 0.07\) mmol/mol phenylalanine (\(P = 0.005\)).

### Table 1. Plasma \(m\)-Tyrosine and \(o\)-Tyrosine Concentrations (ng/mL) and Phenylalanine Concentrations (\(\mu g/mL\)) Before and After 7 Days of Treatment With 6.25 mg Carvedilol

<table>
<thead>
<tr>
<th>Subject</th>
<th>(m)-Tyrosine Baseline</th>
<th>(m)-Tyrosine Day 8</th>
<th>(o)-Tyrosine Baseline</th>
<th>(o)-Tyrosine Day 8</th>
<th>Phenylalanine Baseline</th>
<th>Phenylalanine Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.20</td>
<td>4.02</td>
<td>4.41</td>
<td>4.36</td>
<td>11.68</td>
<td>11.43</td>
</tr>
<tr>
<td>2</td>
<td>4.19</td>
<td>3.75</td>
<td>3.93</td>
<td>3.69</td>
<td>10.98</td>
<td>10.93</td>
</tr>
<tr>
<td>3</td>
<td>3.90</td>
<td>3.56</td>
<td>5.46</td>
<td>4.69</td>
<td>10.65</td>
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<tr>
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<td>5.77</td>
<td>6.56</td>
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<td>12.56</td>
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<td>10.27</td>
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<tr>
<td>6</td>
<td>4.65</td>
<td>4.55</td>
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<td>11.22</td>
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<td>2.98</td>
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<tr>
<td>8</td>
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<td>2.60</td>
<td>3.40</td>
<td>3.27</td>
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<td>4.24</td>
<td>11.15</td>
<td>11.11</td>
</tr>
<tr>
<td>SD</td>
<td>0.99</td>
<td>0.97</td>
<td>1.10</td>
<td>0.90</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

\((P = 0.01)\) \((P = 0.04)\) \((P = 0.329)\)
phenylalanine changed from 0.37±0.08 to 0.35±0.07 mmol/mol phenylalanine (P=0.002) (Tables 1 and 2).

Discussion

Our data demonstrate clearly that ROS generation by both PMNLs and MNCs is significantly diminished by the administration of carvedilol at a small dose (6.25 mg/d) to normal subjects. One week of carvedilol treatment resulted in a 44% reduction in ROS generation by PMNLs and a 35% reduction in that by MNCs. In contrast, ROS generation by leukocytes in normal subjects not given any drugs did not change significantly over a period of 1 week. Whether other cells in the body also respond to carvedilol by reducing ROS generation is not clear from our study. If this effect of carvedilol is indeed extended to other cells and tissues, the total oxidative load of individuals on carvedilol should diminish markedly after treatment with this drug. This would be reflected in the reduction of oxidative damage to the body as measured by such indices as o- and m-tyrosines. Oxidative damage may be an important mechanism underlying several pathophysiological states, eg, atherosclerosis due to oxidative modification of LDL,10; diabetic complications due to oxidative damage of lipids, proteins,11 and DNA,12; aging due to oxidative damage of proteins; and myocardial damage/loss through oxidative injury.

Our data also demonstrate that o-tyrosine and m-tyrosine concentrations fall without a change in phenylalanine concentrations. Because o- and m-tyrosine are formed by ROS attack on phenylalanine, our data indicate that ROS-induced oxidative damage to amino acids and proteins falls in association with the decline in ROS generation by PMNLs and MNCs. This has implications for cellular and extracellular proteins, including enzymes, and their physiological functions. It is possible that apoprotein and lipoprotein molecules may also be involved in ROS-induced damage.

The magnitude of ROS inhibition by MNCs and PMNLs (35% and 44%) was comparable to that observed after administration of vitamin E (1200 IU/d) for 8 weeks, a reduction of ∼50% in superoxide radical generation and H2O2 production by monocytes.13

Carvedilol has been shown to improve outcomes in congestive heart failure by reducing morbidity and mortality and the rate of hospital admissions.1 Although the reduction in sudden death in such patients may be a function of the antiarrhythmic effects of carvedilol, the reduction of deterioration of congestive heart failure may be due to its antioxidant effects, possibly through the protection of the myocardium from ROS damage.

The mechanism underlying this inhibitory effect of carvedilol on ROS generation is not clear from our data. Carvedilol has been shown to possess antioxidant properties in various animal models.9,10 The experimental data have been focused on carvedilol as a chemical antioxidant. There is only 1 report based on a human study, which demonstrates that the ex vivo oxidizability of LDL prepared from sera of patients treated with carvedilol is significantly diminished.11 Our assay system determines actual ROS generation by leukocytes, thus focusing on the biological antioxidant property of carvedilol. It is possible that carvedilol exerts its antioxidant effect by both chemical and biological mechanisms.

In conclusion, we have demonstrated that carvedilol inhibits ROS generation by PMNLs and MNCs significantly, even after a short-term treatment at a relatively small dose. This reduction in ROS generation probably contributes to the diminished conversion of phenylalanine to o- and m-tyrosine and to the previously described antioxidant effects and related clinical benefits.

Acknowledgments

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References

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