Carvedilol Reduces Plasma 8-Hydroxy-2'-Deoxyguanosine in Mild to Moderate Hypertension
A Pilot Study

Juyong Lee, Mejeong Lee, Jeong-Uk Kim, Kyung Il Song, Yun Seok Choi, Sang-Sig Cheong

Abstract—The purpose of this pilot study was to test whether carvedilol has a protective effect against oxidative deoxyribonucleic acid (DNA) damage in human hypertension in vivo. Carvedilol’s antioxidant effect has mostly focused on lipid or amino acid so far. However, there has been no data that carvedilol reduces DNA damage in human hypertension. Never-treated mild to moderate hypertension patients and age- and sex-matched control subjects volunteered for the study. The hypertension subjects were given 12.5 or 25 mg of carvedilol or hydrochlorothiazide orally for 2 months and controls were not given any. Fasting blood samples were collected before and after carvedilol. Plasma highly sensitive 8-hydroxy-2'-deoxyguanosine (hs8-OHdG) and high-sensitivity C-reactive protein (hsCRP) were checked with the samples. There were no statistical differences in clinical characteristics in 3 groups. The hs8-OHdG declined from 9.07 ± 4.23 ng/mL to 5.74 ± 3.89 ng/mL (P = 0.002) after carvedilol. However, it did not show significant reduction after hydrochlorothiazide (9.01 ± 3.89 versus 8.23 ± 4.12 ng/mL; P = NS). In the control group, the hs8-OHdG concentration was 3.41 ± 2.03 ng/mL and 3.01 ± 2.65 ng/mL at baseline and 2 months later, respectively (P = NS). The baseline hs8-OHdG levels were higher in hypertension groups compared with control (P = 0.000). The hsCRP had no significant difference before and after the tested drugs in 2 hypertension groups (group A: 0.21 ± 0.51 versus 0.19 ± 0.37 mg/dL; group B: 0.20 ± 0.45 versus 0.18 ± 0.42 mg/dL). In conclusion, DNA damage caused by reactive oxygen species occurs more in the hypertension patients than normals. Carvedilol significantly reduces DNA damage in the hypertension patients. (*Hypertension. 2005;45:986-990.*)

Key Words: antioxidants

Reactive oxygen species (ROS) generation can result in modification of proteins, lipids, and DNA. It has been suggested that carvedilol has antioxidant actions that synergizes with its nonspecific beta- and alpha-blocking effects. Among the active metabolites of carvedilol, 1-hydroxy-carbazole and 3-hydroxy-carbazole have 50- to 80-fold more potent antioxidant effects than carvedilol itself. The antioxidant effect of carvedilol has been evaluated and characterized in detail in a variety of in vitro test systems and in a broad spectrum of animal models so far. For instance, carvedilol inhibits lipid peroxidation in myocardial cell membrane and inhibits formation of oxidized low-density lipoprotein, protects endothelial cells and vascular smooth muscle cells, and inhibits oxidative amino acid damage (phenylalanine). Carvedilol also has an inhibitory effect on superoxide ion release from activated neutrophils and preserves endogenous antioxidant systems (vitamin E, glutathione). Deoxyguanosine is one of the constituents of DNA. When it is oxidized, it is altered into 8-hydroxy-2'-deoxyguanosine (8-OHdG). The plasma levels of the DNA repair product, highly sensitive 8-OHdG (hs8-OHdG), have been proposed as a noninvasive biomarker of oxidative DNA damage in humans in vivo.

Most of the studies of carvedilol’s antioxidant effect have focused on cellular lipid or amino acid so far. However, there has been no data that carvedilol reduces DNA damage in human hypertension. Therefore, we undertook this study to investigate the effect of carvedilol administration against oxidative DNA damage in humans in vivo.

Methods
Between April 2002 and July 2002, we enrolled 38 patients with newly diagnosed mild to moderate hypertension and who had not used antihypertensive medication previously and 22 age- and sex-matched control subjects with normal blood pressure into a prospective registry. The institutional review board of human research of the Gangneung Asan Hospital approved the study protocol. Patients agreed to participate in this double-blind randomized study and written informed consent was obtained from each subject. None of the subjects was using any medications, including nonsteroidal...
anti-inflammatory drugs, vitamin E, or other antioxidants. Blood pressure measurement methods followed the Joint National Committee 7 guidelines.11

Fasting blood samples were collected at baseline in tubes with ethylenediaminetetraacetic acid as an anticoagulant. The hypertension patients were assigned to carvedilol (group A) or hydrochlorothiazide (group B) according to a randomization list to differentiate the renal DNA protective effect of carvedilol from blood pressure-lowering effect itself. The department of pharmacology blinded medication. The subjects of group A (n=17; mean age, 51±11) and group B (n=21; mean age, 56±13) were given 12.5 to 25 mg of carvedilol or 12.5 to 25 mg of hydrochlorothiazide orally once per day for 2 months, respectively. The references of carvedilol doses (12.5 to 25 mg/d) that we gave to the patients for the antioxidants effects were selected according to the previous reports.7,12 The dose selection, either 12.5 or 25 mg of carvedilol and either 12.5 or 25 mg of hydrochlorothiazide, for each individual patient, was left to the discretion of the coordinator of this study based on the initial blood pressure level. Two months later, another blood sample was collected as described. Age- and sex-matched control subjects (n=22; mean age, 45±12) not given any drugs also had 2 samples taken 2 months apart. Blood samples were separated to plasma immediately and stored in a −97°C refrigerator.

Measurement of Plasma hs8-OHdG and High-Sensitivity C-Reactive Protein

hs8-OHdG was measured with enzyme-linked immunosorbent assay (ELISA) method following the maker’s manuals (Japan Institute for Control of Aging). Briefly, ELISA was conducted on polystyrene 96-well flat-bottom plates (Nunc-ImmunoPlate Maxisorb) using the hs8-OHdG ELISA kit. A monoclonal IgG specific for hs8-OHdG was used in this ELISA in combination with horseradish peroxidase-conjugated anti-mouse polyclonal IgG and substrate o-phenylenediamine. The absorbency was measured at 492 nm using a computerized ELISA reader (MPR A4; Toyo Soda). The concentrations of hs8-OHdG in the test samples were interpolated from the standard curve using log transformation. The lower limit of detection for hs8-OHdG was 0.125 ng/mL. The ELISA test was performed 3 times for 1 sample and the average of 3 was used as a test result. High-sensitivity C-reactive protein (hsCRP) was also checked 2 times by Immuno Nephelometry (Behring). The intra-assay and interassay coefficients of variation were 3.1% and 2.5% for hsCRP, respectively. The lower limit of detection for hsCRP was 0.0175 mg/dL.

Statistical Analysis

All data are expressed as mean±SD. The concentrations of hs8-OHdG and hsCRP were compared with by analysis of variance with post-hoc 3-way comparisons by the Bonferroni–Dunn test, correcting for multiple comparisons among 3 groups. Statistical comparison of the concentration of hs8-OHdG at baseline and at month 2 in each group was performed by Wilcoxon signed rank test after testing with 1-sample Kolmogorov–Smirnov test.

Results

There were no statistical differences in age, sex, body mass index, smoking, diabetes, and total cholesterol level among all 3 groups except initial blood pressure (Table). Follow-up blood pressure after 2 months of treatment showed similar blood pressure reduction effect in group A (carvedilol) and group B (hydrochlorothiazide) (Table).

Baseline plasma hs8-OHdG concentration of 3 groups was 9.07±4.23 (group A), 9.01±3.89 (group B), 3.41±2.03 (group C) ng/mL, respectively, showing that hypertension group had significantly higher hs8-OHdG levels than control group (P=0.002) (Figure 1). After 2-month treatment of carvedilol, there was a significant reduction of hs8-OHdG concentration from 9.07±4.23 to 5.74±3.89 ng/mL (P=0.01) in group A. However, in groups B (hydrochlorothiazide) and C (control group), the hs8-OHdG concentration did not change significantly before and after treatment (group B: 9.01±3.89 versus 8.23±4.12 ng/mL, P=NS; group C: 3.41±2.03 versus 3.01±2.65 ng/mL, P=NS) (Figure 1). Baseline and follow-up concentration of hs8-OHdG comparatively showed in each individual of group A treated with carvedilol a concordant reducing trend of the concentration (Figure 2).

Hypertension groups (groups A and B) had higher hsCRP levels than normal controls (group C) (Figure 3). The hsCRP concentration did not show significant reduction before and after treatment in group A (0.21±0.51 versus 0.19±0.37 mg/dL; P=NS) and group B (0.20±0.45 versus 0.18±0.42 mg/dL; P=NS) (Figure 3).

Discussion

Our data clearly demonstrate that oxidative DNA damage occurs in hypertensive patients more than in normal blood pressure subjects and that the oxidative DNA damage is

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tr>
<td>Age, y</td>
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<td>150/95</td>
<td>115/75</td>
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<td>125/85</td>
<td>120/80</td>
<td>NS</td>
</tr>
<tr>
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<td>9/17</td>
<td>9/21</td>
<td>12/22</td>
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<td>DM</td>
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<td>1/21</td>
<td>0/22</td>
<td>NS</td>
</tr>
<tr>
<td>T chol</td>
<td>206±40</td>
<td>198±37</td>
<td>206±40</td>
<td>NS</td>
</tr>
</tbody>
</table>

BP indicates blood pressure (mm Hg); BMI, body mass index (kg/m²); DM, diabetes mellitus; F, female; M, male; T chol, total cholesterol (mg/dL).

No significant difference in clinical features of 3 groups except baseline BP.
significantly diminished by the administration of carvedilol at the therapeutic dose lowering blood pressure (12.5 to 25 mg/d). The 2-month treatment of carvedilol resulted in a 36.7% reduction in oxidative damage. Distinct from this, the reduction of hs8-OHdG did not occur in the treatment of hydrochlorothiazide group, which had the same blood pressure reduction. Therefore, we were convinced that the protective effect of DNA damage from hypertension might have not related to the blood pressure lowering, but it might have been the sole effect of carvedilol in this study. The level of hs8-OHdG of normal subjects who are not given any drug also did not show significant change over a period of 2 months. Therefore, we, for the first time to our knowledge, demonstrated the beneficial effect of carvedilol on oxidative DNA damage in human in vivo.

Oxidative damage may be an important mechanism underlying several pathophysiological states, for example, atherosclerosis caused by oxidative modification of low-density lipoprotein; diabetic complications caused by oxidative damage of lipids, proteins, and DNA; aging caused by oxidative damage of proteins; and myocardial damage/loss through oxidative injury. Regarding hypertension, oxygen free radicals contribute to either the causes or the consequences of hypertension. There have been data regarding how increased ROS formation was mediated in hypertension. Various abnormalities of ion transport in hypertensive individuals enhance contractile response, hypertrophy, and proliferation of vascular smooth muscle cells. Medial thickening of arterial wall is to increase the distance required for diffusion of oxygen from the lumen. A decrease in P02 would result in incomplete oxidation and probably lead to increased concentrations of free radicals and abnormalities of the redox state. There is other evidence that hypertension induces oxidative stress in the arterial wall. Superoxide anions might trigger the development of hypertension in some models, presumably by inactivating endothelium-derived nitric oxide and thus mitigating the important vasodilator mechanism. Xanthine oxidase inhibitors also reduce blood pressure. These observations suggest that xanthine oxidase may be one potential source of the oxygen free radicals. Human copper/zinc superoxide dismutase bound vascular endothelial cells when injected intravenously and localized within the vessel wall, reducing blood pressure in spontaneously hypertensive rats.

The mechanism underlying inhibitory effect of carvedilol on oxidative DNA damage is not clearly shown in these data. Hypertensive patients had significantly higher baseline serum hsCRP than normotensive controls, and CRP has been considered as an independent risk factor for essential hypertension in a previous report. In our study, we also demonstrated that hsCRP was higher in hypertension patients than in normals. However, in our data, hsCRP concentration revealed no significant change before and after carvedilol, considering that the protective effect of carvedilol against oxidative DNA damage probably was not associated with anti-inflammatory effect. On the contrary, one antihypertensive agent, such as candesartan, reduced hsCRP significantly. In that report, the anti-inflammatory effect of candesartan, which means decreased hsCRP, was not correlated with a blood pressure-lowering effect. Therefore, carvedilol might have somewhat different mechanism as an antioxidant than angiotensin receptor blocker. There have been some data focused on carvedilol as a chemical antioxidant. Recently, Dandona et al demonstrated an inhibitory effect of carvedilol on ROS generation by leukocyte and oxidative DNA damage to amino acids in humans in vivo. They showed a biological antioxidi-
A standardized commercial assay that does not require special sides, 8-OHdG was selected as a marker of oxidative DNA damage in this study because of its convenient use in clinical practice. Monitoring of ROS formation on several targets in vivo has focused on the biomarkers of oxidative stress in various studies so far. Essentially, the approach has been indirect and configured on the identification of chromosomally stable, free radical-catalyzed products of lipid peroxidation (such as isoprostanes), modified proteins (such as nitrated fibrinogen), antibodies directed against oxidation-dependent epitopes in low-density lipoprotein, and indices of free radical-catalyzed modification of DNA (such as 8-OHdG). Among these oxidative damages in the human body, DNA damage is involved in addition to reactions by free radicals, leading to carbon-centered sugar radicals and OH- adduct or H- adduct radicals of heterocyclic bases of DNA. There are some techniques for the detection of DNA damage such as gas chromatography or high-performance liquid chromatography with mass spectrometry, which identify and accurately quantify modified nucleosides. However, it is difficult to use these techniques practically for clinical purposes because of the technical complexity. On the contrary, as the indicator of oxidative DNA damage, measuring of hs8-OHdG is easy to use in a cost-effective manner even in an outpatient setting. It is also a standardized commercial assay that does not require specialized plasma collection or assay technique. Hs8-OHdG is a biomarker substance that aids detection of oxidative DNA damage sensitively and proportionally, responding to the degree of oxidative stress caused in the body. It is stable in human body after it is excreted from DNA by the repair enzyme system, and is ultimately released into blood and excreted via urine. Measuring the concentration of hs8-OHdG with ELISA to determine the total oxidative stress in the body is excreted via urine. Measuring the concentration of hs8-OHdG showed that the monoclonal antibody did not cross-react with the original 4 deoxyribonucleosides, other DNA base-modified products such as 8-hydroxy-2’-deoxyadenosine and 8-methyl-2-deoxyguanosine, or urine components such as uric acid, creatine, and creatinine. So far, urinary samples have been more frequently used for 8-OHdG tests. However, we used plasma samples, not urine, for checking 8-OHdG because we thought that the test result of random urine might not directly show the real data for 8-OHdG, although it is compensated for with the result of creatinine excretion rate. Among urine sample methods, 24-hour collected urine is better than random urine sample methods. However, it may be difficult to collect all the urine for 24 hours exactly in routine clinical settings if this test is widely used in the future.

Isoprostanes, such as the lipid (arachidonic acid) peroxidation metabolite, have been emerged as one of the best indices of oxidative injury. However, we used hs8-OHdG as the marker of oxidative DNA damage in this study, because we focused on the oxidative DNA damage, but not lipid oxidation or general oxidative damage. The good correlation between 8-OHdG and isoprostanes was well-documented in one report. However, there have been some controversies with isoprostanes. In a study with rats, it was tested whether coffee or chlorogenic acid inhibits 8-OHdG in vivo and in vitro. 8-OHdG and isoprostane were both checked. It was shown that 8-OHdG was significantly increased in a dose-dependent manner. However, isoprostane levels did not show changes. In another study of never-treated mild-to-moderate hypertension, lipid peroxidation, ie, isoprostane level, was not increased. Therefore, for the detection of DNA damage, not for general oxidative stress or lipid oxidation, we are convinced that hs8-OHdG is better than isoprostanes.

In conclusion, it is likely that DNA damage caused by ROS occurs more in the hypertension patients than in persons with normal blood pressure, and that carvedilol significantly reduces DNA damage in hypertension in vivo. A future investigation evaluating the mechanism of carvedilol’s protective antioxidant interaction with damaged DNA and a large clinical trial might be needed.

**Perspectives**

Because humans live longer than before, hypertension may become the most troublesome problem. Therefore, we should focus on the prevention of development of hypertension as well as on the reduction of the complication of hypertension. Oxidative damage is closely related to the development and complications of hypertension. Furthermore, hypertension is one of the most important risk factors of atherosclerosis. Oxidative DNA damage also should be importantly considered because it may change genes and result in problems for offspring of those with DNA damage. Therefore, researchers need to develop other antioxidant antihypertensive medications. Among the many antihypertensive drugs, there might be some drugs that have antioxidant effects, like carvedilol. This report is important because it clearly shows that the antioxidant antihypertensive drug, carvedilol, decreases oxidative DNA damage. Doctors do not know in their daily practice who might have more serious oxidative damage, especially DNA damage, among hypertension patients. Therefore, we need simple, accurate, and specific test kits like hs8-OHdG for oxidative DNA damage so that we can use the appropriate drug, such as carvedilol, among many other antihypertensive medications, for hypertension patients who have more DNA damage.
References


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