Factors Related to the Impact of Antihypertensive Treatment in Antioxidant Activities and Oxidative Stress By-Products in Human Hypertension

Guillermo T. Sáez, Carmen Tormos, Vicente Giner, Javier Chaves, Jose V. Lozano, Antonio Iradi, and Josep Redón

The objective was to study factors related to the changes induced by antihypertensive treatment on oxidative status, antioxidant activities, and reactive oxygen species by-products in whole blood and mononuclear peripheral cells. Eighty-nine hypertensive patients (mean age 46 years, 46 men, average 24-h blood pressure 139/88 mm Hg, body mass index 29) were included. After 3 months of nonrandomized allocation to antihypertensive treatment (20 nonpharmacologic, 36 β-blockers, 33 angiotensin receptor blocker), oxidized/reduced glutathione ratio and malondialdehyde were significantly reduced, and the activity of superoxide dismutase, catalase, and glutathione peroxidase was significantly increased in both whole blood and peripheral mononuclear cells. The content of damaged base 8-oxo-2'-deoxyguanosine in nuclear and mitochondrial DNA in hypertensive subjects was also significantly reduced during the antihypertensive treatment. In a group of 42 subjects, the oxidative stress was further reduced and the antioxidant enzyme activities further increased after 12 months of antihypertensive treatment. The changes were independent of the kind of antihypertensive treatment. In conclusion, antihypertensive treatment improved the increased oxidative stress and the decreased antioxidant mechanisms. It is independent of the type of treatment and the beneficial effect of treatment increases over time.

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Key Words: Oxidative stress, hypertension, antioxidants, DNA damage, antihypertensive treatment.

Hypertension is considered a state of oxidative stress that can contribute to the development of atherosclerosis and other hypertension-induced organ damage. An excessive production of reactive oxygen species (ROS), outstripping antioxidant defense mechanisms, has been implicated in pathophysiologic conditions impacting on the cardiovascular system. Assessment of antioxidant activities and lipid peroxidation by-products in hypertensives indicates an excessive amount of ROS and a reduction of antioxidant mechanism activity in blood as well as in several other cellular systems, including not only vascular wall cells but also those found in circulating blood.

Antihypertensive treatment aims to reduce not only blood pressure (BP) values but also hypertension-induced organ damage and as a consequence morbidity and mortality. Previous reports demonstrated that lowering BP leads to a reduction of oxidative stress. These studies, however, used a low number of ROS parameters and no information was provided about potential factors related to the oxidative stress reduction, such as the impact of different antihypertensive treatment and the influence of the duration of treatment.

In the present study, oxidative status, antioxidant activities, and ROS by-products in whole blood and mononuclear peripherals cells were measured before and at 3 and 12 months of antihypertensive treatment in a group of hypertensive subjects in whom BP values were estimated by using 24-h ambulatory BP monitoring. Furthermore, factors related to the changes observed were analyzed.

Methods
Selection of Study Participants

Patients included in the study were selected from an out-patient clinic during the 1-year period from January to
December 2001. Patients who fulfilled the inclusion criteria were invited to participate, and written consent was requested. The following were the inclusion criteria: 1) essential hypertension defined according to the criteria of the sixth Joint National Committee; 2) aged 25 to 50 years; 3) World Health Organization grade I to II; and 4) never previously been treated for hypertension. Patients with diabetes mellitus, a fasting glucose in serum of >120 mg/dL, with total cholesterol levels >240 mg/dL, or cigarette consumption >10 per day were excluded. The Hospital’s Ethics Committee approved the study, and all participants gave informed written consent to participate in this protocol.

Study Design
After enrollment, patients were given usual care treatment, which included nonpharmacologic treatment, consisting of moderate salt restriction and a low-calorie diet if overweight. Nonpharmacologic treatment alone was maintained in patients who had an average of awake ambulatory BP of <135/85 mm Hg. The remaining patients were randomized to receive a course of antihypertensive drugs based on β-blockers (atenolol, bisoprolol) or an angiotensin receptor blocker (ARB) (telmisartan). Hydrochlorothiazide was added where necessary to maintain the BP goal of <135/85 mm Hg. In half of the patients, the follow-up period was extended to 1 year. Blood pressure measurements and blood samples for analytical and oxidative stress parameters were obtained at the beginning, before the antihypertensive treatment, and at 3 and 12 months during the antihypertensive treatment.

BP Measurements
Blood pressure was measured using a mercury sphygmomanometer with the patient in the sitting position after 5 min of rest in a quiet environment, following the recommendations of the British Hypertension Society. Systolic BP, diastolic BP (Korotkoff phase I and phase V, respectively) and mean BP (MBP) were averaged using three readings measured at 5-min intervals. Ambulatory BP monitoring was performed using an oscillometric monitor (Spacelabs 90202 or 90207, Redmond, WA) on a regular work day at the time of the initial evaluation and after 3 and 12 months of antihypertensive treatment. Following the standard protocol, recording began between 8:30 and 9 AM with readings taken every 20 min from 6 AM until midnight and every 30 min from midnight to 6 AM. Before starting the study, the reliability of the BP values measured with the monitor was checked against simultaneous measurements by a mercury sphygmomanometer. Differences of less than 5 mm Hg were allowed. Those patients with recordings showing an error rate of >25% of the total readings were excluded from the study.

Analytical Procedures
Blood samples were obtained in the morning after a minimum of 8 h of fasting. Serum biochemical profiles were measured using an autoanalyzer. Levels of glutathione reduced (GSH), glutathione oxidized (GSSG), malondialdehyde, genomic, and mitochondrial 8-oxodeoxyguanosine (8-oxo-dG), and the activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were assessed as previously described.

In brief, levels of GSH, GSSG, and malondialdehyde (MDA) were measured after extraction by using high performance liquid columns (HPLC) in blood and in mononuclear cells isolated by Ficoll-Hypaque centrifugation. Activity of the antioxidant enzymes, SOD, catalase, and GPx were measured by inhibition of substrate oxidation. The amount of 8-oxo-dG and deoxyguanosine (dG) in the DNA digest was measured by electrochemical and UV absorbency detection after genomic and mitochondrial DNA separation. Mitochondrial DNA was extracted as described by Latorre et al using 200 μL of mononuclear cell suspension treated at the precise step with 120 μL of potassium acetate 3 M at pH 4.8 and stored at −20°C for 20 min to precipitate high molecular weight nuclear DNA and proteins. Mitochondrial DNA was then extracted with an equivalent volume of isopropanol and 10 min of centrifugation at 13,000 rpm. The supernatant was transferred to a new eppendorf and mixed with an equivalent volume of isopropanol and stored at room temperature for 5 min. After centrifugation at 13,000 rpm for 10 min, the obtained pellet, containing mitochondrial DNA was washed with 70% ice-cold ethanol and stored at −20°C until used or resuspended with 150 μL Tris/EDTA buffer at pH 8.

Statistical Analysis
For each variable, the values were expressed as mean ± standard deviation or standard error values. We calculated the intra-assay reproducibility for the oxidative stress parameters using the Bland and Altman method in a group of 12 subjects. The coefficients of repeatability, expressed as a percentage of the nearly maximal variation, namely 4 standard deviation of the measurement under investigation, were as follows: GSH 2%; activity of SOD, CAT, and GPx ranged from 2% to 8%; and for MDA 15%, genomic DNA 23%, and mitochondrial DNA 11% (the lower the value, the higher the reproducibility). Changes in the analyzed parameters from the start to the third month and twelfth month of treatment were sought by the t paired test. The differences in BP, oxidative stress parameters, and by-products among the treatment groups were analyzed by a two-way ANOVA with repeated measurements. The sample size was able to demonstrate a 20% difference among groups with an α-error of 5% and a minimal statistical error of 80%. The relationship between changes in BP and the oxidative stress-derived parameters was assessed using Pearson's
Creatinine (mg/dL) 0.86
Triglycerides (mg/dL) 129.6
LDL cholesterol (mg/dL) 135.1
HDL cholesterol (mg/dL) 50.3
Total cholesterol (mg/dL) 213.4
Glycosylated Hb (%) 5.01
Baseline glucose (mg/dL) 101.1

chlorothiazide treatment. The characteristics of the pa-

RESULTS

General Characteristics of the Study Population

The study was performed on 89 hypertensives. After the

Table 1. General characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nonpharmacologic (n = 20)</th>
<th>β-blockers (n = 36)</th>
<th>ARBs (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>44.1 ± 9.3</td>
<td>47.1 ± 10.7</td>
<td>46.1 ± 9.9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/11</td>
<td>20/16</td>
<td>17/16</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.2 ± 4.6</td>
<td>29.8 ± 4.2</td>
<td>29.0 ± 3.7</td>
</tr>
<tr>
<td>Baseline glucose (mg/dL)</td>
<td>101.1 ± 15.4</td>
<td>101.9 ± 10.4</td>
<td>100.6 ± 9.6</td>
</tr>
<tr>
<td>Glycosylated Hb (%)</td>
<td>5.01 ± 0.37</td>
<td>4.99 ± 0.34</td>
<td>4.85 ± 0.41</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>213.4 ± 31.6</td>
<td>217.6 ± 30.6</td>
<td>210.0 ± 29.8</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>50.3 ± 8.7</td>
<td>46.7 ± 9.7</td>
<td>45.6 ± 9.4</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>135.1 ± 35.6</td>
<td>141.8 ± 31.4</td>
<td>139.2 ± 28.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>129.6 ± 67.3</td>
<td>142.4 ± 58.9</td>
<td>138.7 ± 68.2</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.86 ± 0.14</td>
<td>0.90 ± 0.13</td>
<td>0.92 ± 0.17</td>
</tr>
</tbody>
</table>

ARBS = angiotensin receptor blockers; Hb = hemoglobin.
Values are expressed as mean ± standard deviation.

correlation coefficients. Two-tailed values of \( P < .05 \) were considered as statistically significant.

Oxidative Stress and Antihypertensive Treatment

After 3 months of antihypertensive treatment, office and the average of 24-h ambulatory systolic and diastolic BP decreased significantly in the 89 subjects (Table 2). Concurrently with the decrease in BP, there was a significant reduction of the GSSG levels and an increase of the GSH in blood and in the mononuclear cells, increasing the GSH/GSSG ratio (Table 3). Likewise, a significant decrease in the MDA, a by-product of lipid peroxidation, was also observed.

Table 2. Office and ambulatory blood pressure (BP) at the beginning and after 3 months of antihypertensive treatment in the study population grouped by kind of treatment

<table>
<thead>
<tr>
<th></th>
<th>Nonpharmacologic (n = 20)</th>
<th>β-blockers (n = 36)</th>
<th>ARBs (n = 33)</th>
<th>Total (n = 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>151.3 ± 4.5</td>
<td>159.8 ± 2.8</td>
<td>162.5 ± 3.5</td>
<td>158.5 ± 2.0</td>
</tr>
<tr>
<td>( P )</td>
<td>.004</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3 month</td>
<td>135.8 ± 4.7</td>
<td>136.7 ± 2.9</td>
<td>133.7 ± 3.6</td>
<td>134.7 ± 2.0</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>95.9 ± 2.6</td>
<td>96.8 ± 1.7</td>
<td>101.3 ± 2.2</td>
<td>97.6 ± 1.1</td>
</tr>
<tr>
<td>( P )</td>
<td>.484</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3 month</td>
<td>91.1 ± 4.1</td>
<td>81.1 ± 2.6</td>
<td>79.3 ± 3.4</td>
<td>83.6 ± 1.7</td>
</tr>
<tr>
<td>24-hour ambulatory BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>129.1 ± 3.7</td>
<td>141.9 ± 2.4</td>
<td>140.9 ± 2.8</td>
<td>138.3 ± 1.6</td>
</tr>
<tr>
<td>( P )</td>
<td>.330</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3 month</td>
<td>127.8 ± 3.6</td>
<td>129.5 ± 2.3</td>
<td>122.1 ± 2.7</td>
<td>126.6 ± 1.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>81.9 ± 2.4</td>
<td>88.9 ± 1.5</td>
<td>90.7 ± 1.8</td>
<td>87.6 ± 1.0</td>
</tr>
<tr>
<td>( P )</td>
<td>238</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3 month</td>
<td>80.9 ± 1.5</td>
<td>80.3 ± 1.4</td>
<td>77.3 ± 1.7</td>
<td>79.3 ± 0.9</td>
</tr>
</tbody>
</table>

ARBS = angiotensin receptor blockers; DBP = diastolic BP; SBP = systolic BP.
Values are expressed as mean ± standard error adjusted by age and sex.
\( P \) values denote differences between hypertensives and controls.
In mononuclear cells, 8-oxo-dG from genomic and mitochondrial DNA decreased 3 months after the start of treatment. Activity of the antioxidant enzymes tested, SOD, catalase, and GPx, increased in both blood and mononuclear cells during the antihypertensive treatment (Table 4). During the antihypertensive treatment there was no significant change in the levels of fasting glucose (baseline 101.5 mg/dL, on treatment 101.4 mg/dL), glycosylated hemoglobin (HbA1c) (baseline 4.98%, on treatment 4.95%), total cholesterol (baseline 213.5 mg/dL, on treatment 210.1 mg/dL), LDL cholesterol (baseline 137.7 mg/dL, on treatment 132.5 mg/dL), triglycerides (baseline 129.1 mg/dL, on treatment 130.8 mg/dL), or creatinine level (baseline 0.88 mg/dL, on treatment 0.89 mg/dL).

Despite the concomitant decrease in BP and the reduction in oxidative stress, there was no significant correlation between the parameters. Pearson correlation coefficients between the decrease in the average of 24-h MBP and the changes in the parameters assessed were: blood GSSG/ GSH r = 0.02 (P = .88); blood MDA r = −0.16 (P = .33).
Whether or not the kind of antihypertensive treatment influences the changes of oxidative stress analyzed in the overall population. No significant differences in terms of age, body mass index, or sex distribution were observed among groups. At the beginning, the office systolic and diastolic BP and the average of 24-h systolic and diastolic BP were significantly lower in the group that received nonpharmacologic treatment when compared to the other two groups (office systolic BP \( P = .01 \); office diastolic BP \( P = .04 \); 24-h ambulatory systolic BP \( P < .001 \); 24-h ambulatory diastolic BP \( P < .001 \)), whereas no difference was observed between the \( \beta \)-blocker and ARB groups (Table 2). Blood pressure reduction in the \( \beta \)-blocker or ARB treatment groups was significantly higher than that in the nonpharmacologic treatment group.

Parameters of the oxidative stress (Table 3), and of the antioxidant mechanisms (Table 4) were not different across the three treatment groups at the beginning of the study. After 3 months of therapy, a significant reduction of oxidative stress parameters and a significant increase in the antioxidant mechanisms were observed in all treatment groups. Using a two-way variance analysis no difference was observed among the treatment groups in terms of changes in blood or mononuclear cells of GSSG, GSH, GSSG/GSH ratio, MDA, and the enzymatic activity of the antioxidant enzymes SOD, catalase, and GPx. Likewise no difference was observed in the levels of genomic or mitochondrial 8-oxo-dG in mononuclear cells.

From these results we can conclude that the type of antihypertensive treatment did not exert a major impact in the reduction of the oxidative stress.

### Influence of the Antihypertensive Treatment Type

Whether or not the kind of antihypertensive treatment influences the changes of oxidative stress was analyzed in the overall population. No significant differences in terms of age, body mass index, or sex distribution were observed among groups. At the beginning, the office systolic and diastolic BP and the average of 24-h systolic and diastolic BP were significantly lower in the group that received nonpharmacologic treatment when compared to the other two groups (office systolic BP \( P = .01 \); office diastolic BP \( P = .04 \); 24-h ambulatory systolic BP \( P < .001 \); 24-h ambulatory diastolic BP \( P < .001 \)), whereas no difference was observed between the \( \beta \)-blocker and ARB groups (Table 2). Blood pressure reduction in the \( \beta \)-blocker or ARB treatment groups was significantly higher than that in the nonpharmacologic treatment group.

### Influence of the Antihypertensive Treatment Duration

The impact of the duration of the oxidative stress parameters was analyzed in 42 subjects (7 nonpharmacologic...
treatment, 20 β-blocker, 15 ARB) in whom additional assessment was performed at the end of the 12 months of antihypertensive treatment. The characteristics of this group did not differ from the total study population. After 1 year of treatment, the office and the average of ambulatory systolic and diastolic BP were not different from those observed at 3 months of treatment. In the oxidative stress parameters, which were reduced at 3 months of treatment, a further and significant reduction during the extended follow-up for GSSG/GSH, MDA, genomic, and mitochondrial 8-oxo-dG was observed. In Fig. 1 some of the most representative values are presented. The increment in the antioxidant enzymatic activity was also further enhanced at the first year of treatment for SOD and catalase activity, as compared to the values observed at 3 months (Fig. 1). Only glutathione peroxidase activity in both blood and intracellular maintained the same values that were present at both 3 and 12 months.

Looking at the data presented, the greater the duration of treatment, the better the reduction in oxidative stress and the greater the increase in the antioxidant mechanisms.

Discussion
We simultaneously examined the activities of the most important antioxidant enzymes SOD, CAT, and GPx and the levels of GSH, GSSG in blood and in the mononuclear peripheral cells from hypertensive patients before and after antihypertensive treatment. Malondialdehyde and 8-oxo-dG from lipid peroxidation and DNA modification, respectively, were also assessed. The results obtained indicate that blood and peripheral mononuclear cells from hypertensive patients, which exhibit important deficiencies of physiologic antioxidants and large quantities of lipid peroxidation and DNA oxidation by-products accumulated, improved during the antihypertensive treatment. Antihypertensive treatment by itself decreased oxidative stress status, as well as the ROS by-products, and increased antioxidant mechanisms. It seems that changes were not dependent on the extent of BP reduction or the type of antihypertensive treatment. Extending treatment overtime further improved the oxidative stress parameters.

A number of methods have been used to assess oxidative stress in biological systems. The methods used in the present study analyzed the bioavailability of the most important antioxidant mechanisms including GSH, SODs, CAT, and GPx, together with the oxidation by-products MDA and 8-oxo-dG. All are well established in measuring oxidative stress in blood and cells with a low coefficient of intra-assay variability. The analyzed parameters had been selected based on their recognized value as reproducible oxidative stress indicators, as had been recently reviewed.18,19

The growing importance of ROS increases the necessity of reproducible and reliable markers of oxidative stress, and that its assessment is repeatable over time so as to monitor treatment-induced changes. This leads to the measurement of ROS markers in blood or in circulating cells, although the correlation of ROS parameters between circulating and vascular wall cells has not been established. We used mononuclear cells because they play a role in the process of vascular wall inflammation.20 At present it is not clear what type of cells from peripheral blood, if any, best reflect the oxidative stress present in the vascular wall.21

**Fig. 1.** Average of mean blood pressure (24-h MBP), and ratio of glutathione oxidized/reduced (GSSG/GSH), genomic 8-oxo-deoxyguanosine (8-oxo-dG), and superoxide dismutase activity (SOD) in mononuclear cells at baseline and after 3 and 12 months of antihypertensive treatment in 42 patients. **P < .001 statistical difference between baseline and 3 months; **P < .001 statistical difference between 3 and 12 months.
The absence of a linear relationship between the changes in BP values and the oxidative stress parameters is in agreement with the previous observation of our group concerning the absence in the relationship between BP values and the oxidative stress status. This may indicate that factors other than BP values alone may be responsible for the altered oxidative state in blood and in peripheral mononuclear cells of hypertensive patients.

The impact of different types of antihypertensive treatment on oxidative stress has been analyzed in several studies. The design and the outcomes measured make it difficult to establish comparisons among them. In general, the studies were performed during a short period of observation, from 2 to 24 weeks. All of them showed a reduction in oxidative stress and an increase in antioxidant mechanisms after different antihypertensive treatments. In two of the studies differences among the antihypertensive treatments have been observed but not in the others. Taddei et al showed that lacinidipine reduced the generation of LDL hydroperoxides significantly more than the \( \beta \)-blockers. Baykal et al, measuring MDA and SOD activity in blood observed an improvement with angiotensin-converting enzyme inhibitors or ARBs compared with calcium channel blockers, and \( \alpha \)- or \( \beta \)-blockers. In comparison with the present study, the reduction in BP and changes in oxidative stress, among the modalities of treatment we observed did not show significant differences between the nonpharmacologic treatment and the administration of atenolol or telmisartan.

An interesting observation from the present study, not previously assessed, is the time-dependency of the oxidative stress reduction. Extending treatment over time reduced the oxidative stress status, as well as enhancing the antioxidant mechanisms, reaching values that were close to those observed in normotensive controls. This is in agreement with a previous report that measured blood-free radicals sequentially at 4, 8, 12, and 24 weeks during lercanidipine treatment. A progressive reduction in free radicals was observed. It is well known that protection against the development of organ damage in hypertensive patients, although beginning a short time after introducing treatment, increases over time. Whether or not a reduction in oxidative stress and an increment in antioxidant mechanisms indicate protection against vascular damage is an intriguing hypothesis, one that has the most recent support in an article that links the activity of glutathione peroxidase type 1 to the risk of cardiovascular events in a population with coronary heart disease.

In summary, our study reveals that the increased oxidative stress of hypertensive subjects improved with antihypertensive treatment without major differences among the modalities of treatment. Furthermore, extending the treatment over time increases the beneficial impact of treatment. If ROS products from the peripheral mononuclear cells were to contribute to the endothelial dysfunction and to the organ damage present in hypertensive subjects, the improvement during treatment could contribute to the organ damage protection achieved by treatment. Whether or not assessment of oxidative stress may be useful as a guide of cardiovascular risk factors, control is an intriguing hypothesis.

References