Oxidative Stress in Leukocytes Is a Possible Link Between Blood Pressure, Blood Glucose, and C-Reacting Protein

Kenichi Yasunari, Kensaku Maeda, Munehiro Nakamura, Junichi Yoshikawa

Abstract—Because oxidative stress and inflammation are believed to play roles in the pathogenesis of cardiovascular diseases, oxidative stress in polymorphonuclear leukocytes (PMNs) and mononuclear cells (MNCs) has been measured. A total of 529 subjects participated this study. Intracellular oxidative stress in PMNs and MNCs was measured by gated flow cytometry using carboxyfluorescin diacetate bis-acetoxymethyl ester. C-reacting protein (CRP), insulin action (homeostasis model assessment), and traditional risk factors such as age, gender, body mass index, triglycerides, LDL cholesterol, HDL cholesterol, hemoglobin A1c, and mean blood pressure were also measured. Multiple regression analysis revealed a significant correlation between mean blood pressure and PMN oxidative stress (r=0.104, P=0.018). It also demonstrated a significant correlation between hemoglobin A1c and PMN oxidative stress (r=0.112, P=0.021). A significant correlation was also found between CRP and MNC oxidative stress (r=0.116, P=0.008) by multiple regression analysis. In patients with both hypertension and diabetes, both PMN and MNC oxidative stress was increased (n=21, P=0.022 and P=0.006). These results suggest that both hypertension and diabetes lead to increased oxidative stress of PMNs and MNCs, and that CRP is related to MNC oxidative stress. (Hypertension. 2002;39:777-780.)

Key Words: leukocytes □ arteries □ diabetes mellitus □ oxidative stress
Results

Blood Pressure

All subjects were divided into hypertensive group (HT; BP ≥140/90 mm Hg, n = 219) and normotensive group (NT; BP <140/90 mm Hg, n = 310). Baseline characteristics of the 2 experimental groups are shown in Table 1. PMN oxidative stress was significantly increased in cell from the HT group. None of the other differences between measured variables in the 2 groups were significant. The relationship between MBP and oxidative stress in PMNs or MNCs is illustrated in Figure 1 and indicates that the higher the blood pressure, the greater the PMN oxidative stress.

**Blood Glucose (HbA1C)**

All subjects were divided into a diabetes group (DM; HbA1C ≥6.5%, n = 41) or a non-diabetic group (Non-DM; HbA1C <6.5%, n = 488). Baseline characteristics of the 2 experimental groups are shown in Table 2. MNC oxidative stress was significantly increased in the diabetes group. Significant difference was observed between the 2 groups in MNC oxidative stress, HDL cholesterol, HOMA-IR, and insulin (Table 2). The relationship between HbA1C and PMN or MNC oxidative stress was examined by determination of standardized correlation coefficients and multiple regression analysis.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Relationship between MBP and oxidative stress in PMNs (left) or MNCs (right). n = 529

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Relationship between HbA1C and oxidative stress in PMNs (left) or MNCs (right). n = 529
Hypertensive Diabetic Patients

All subjects were divided into 4 groups: NT and Non-DM (n=290), HT and Non-DM (n=198), HT and DM (n=20), and HT and Non-DM (n=21). Baseline characteristics of the 4 experimental groups are shown in Table 3. Only in HT and DM group, were both PMN and MNC oxidative stress significantly increased; there was also an increase in triglycerides and HOMA-IR, an indicator of insulin resistance, and a decrease in HDL cholesterol.

Multiple Regression Analysis

Multiple regression analysis was used to quantify the impact of measured variables on PMN oxidative stress and MNC oxidative stress. The results shown in Table 4 indicate that MBP was significantly related to PMN oxidative stress, and that HbA1c was significantly related to PMN oxidative stress. The results shown in Table 5 indicate that CRP is significantly related to MNC oxidative stress. The relationships between CRP and PMN or MNC oxidative stress are shown in Figure 3. Multiple regression analysis was also used to quantify the impact of measured variables on HOMA-IR, an indicator of insulin resistance. Body mass index (r=0.338, P<0.001, n=529), triglycerides (r=0.149, P=0.004, n=529), and HbA1c (r=0.313, P<0.001, n=529) were significantly related to HOMA-IR. However, neither PMN oxidative stress (P=0.673) nor MNC oxidative stress (P=0.563) was related to HOMA-IR. Oxidative stress in PMNs and MNCs was related (r=0.153, P<0.001, n=529).

Discussion

In the present study, we have demonstrated for the first time that only in hypertensive diabetic patients, both PMN and MNC oxidative stress were significantly increased (Table 3). Both hypertension and diabetes are concurrent, and the

<table>
<thead>
<tr>
<th>TABLE 4. Multiple Regression Analysis of the Relationship Between PMN Oxidative Stress and Other Associated Variables for the Entire Group</th>
<th>TABLE 5. Multiple Regression Analysis of the Relationship Between MNC Oxidative Stress and Other Associated Variables for the Entire Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Factors</td>
<td>Regression Coefficient</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.151</td>
</tr>
<tr>
<td>Gender</td>
<td>6.047</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.651</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>0.636</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.428</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.457</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>-0.018</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>0.017</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>0.092</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>-4.460</td>
</tr>
</tbody>
</table>

Dependent variable is PMN oxidative stress. Independent variables are age, gender, MBP, HOMA-IR, HbA1c, triglycerides, HDL cholesterol, and LDL cholesterol.

R² for entire model=0.35.

Dependent variable is MNC oxidative stress. Independent variables are age, gender, MBP, HOMA-IR, HbA1c, triglycerides, HDL cholesterol, and LDL cholesterol.

R² for entire model=0.38.
relationship with increased cardiovascular events is suggested. Our findings may explain the mechanisms of increased cardiovascular events through the increase in PMN and MNC oxidative stress of hypertensive diabetic subjects.

We have shown that MBP or HbA1C is related to PMN oxidative stress (Table 4), which is consistent with the previous observation. PMNs contain the identical oxidative stress–generating system to endothelial cells. If they are activated, they would be an additional systemic source of oxidative stress.

In the present study, we have also demonstrated for the first time that CRP is related to MNC oxidative stress (Tables 5). CRP has been extensively studied as a potential tool for the prediction of cardiovascular events. Thus, MNC oxidative stress may play a role as a potential tool for prediction of cardiovascular risk.

Risk factors for cardiovascular diseases such as hypertension, diabetes, and hyperlipidemia are related to both oxidative stress and insulin resistance. In the present study, neither PMN oxidative stress nor MNC oxidative stress was directly related to HOMA-IR, an indicator of insulin resistance. Multiple regression analysis showed that body mass index, triglycerides, and HbA1C were related to HOMA-IR, suggesting that obesity-related metabolic abnormality was related to insulin resistance.

Possible mechanism of increase in oxidative stress in PMNs and MNCs in relation to hypertension, diabetes, and CRP remains to be elucidated. Pressure and high glucose are reported to activate protein kinase C activation in vitro, and oxidative stress in PMNs is mediated by protein kinase C. Protein kinase C may play a role in the increase in PMN oxidative stress induced by hypertension and diabetes.

In conclusion, we found a significant relationship between PMN oxidative stress and MBP or HbA1C, in addition to a significant relationship between MNC oxidative stress and CRP. We have identified a possible cellular mechanism to explain why oxidative stress in hypertensive and diabetic individuals are increased.

Acknowledgments
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References
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