Platelet Aggregability in Patients with Hypertension Treated with Angiotensin II Type 1 Receptor Blockers

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**Aim**: Cardiovascular events associated with hypertension often involve thrombosis. Increased platelet activity is one of the risk factors of cardiovascular diseases. Antithrombotic properties of antihypertensive agents are not fully characterized. Angiotensin II type 1 receptor blockers (ARBs) are widely used for the treatment of hypertension. Some ARBs can provoke antiaggregatory effects on platelets in vitro. Whether ARBs can inhibit platelet aggregation was tested in hypertensive patients in vivo.

**Methods**: Platelet aggregation was assessed by the highly sensitive particle counting method using laser-light scattering.

**Results**: Large platelet aggregation induced by adenosine diphosphate (ADP, 3 μM) was 2.6 ± 0.4 (×10^7) (SE) in hypertensive patients treated with losartan (72 ± 3 years old, n = 10) while it was 3.9 ± 0.6 in hypertensive patients treated with candesartan (70 ± 5 years old, n = 6; p = 0.056). Large platelet aggregation induced by thromboxane A2 receptor agonist, U46619 (10 μM), was 2.8 ± 0.5 (×10^7) in hypertensive patients treated with losartan while it was 5.1 ± 0.9 in hypertensive patients treated with candesartan (p = 0.033). Clinical characteristics including the control of blood pressure did not differ between the two groups (losartan 136 ± 5/73 ± 3 mmHg vs. candesartan 135 ± 4/76 ± 5).

**Conclusion**: Thus, losartan may have the possibility to inhibit platelet activation in patients with hypertension independent of blood pressure reduction. Antiaggregatory properties may be independent of angiotensin II type 1 receptor or of antihypertensive actions. The favorable effects of losartan on reduction of adverse cardiovascular events among hypertensive patients may be at least partly mediated by inhibition of platelet activation.

**Key words**: Angiotensin II, High blood pressure, Thrombosis, Thromboxane A2

**Introduction**

Many of the clinical events associated with hypertension are related to thrombosis. Increased platelet aggregability is one of the risk factors for cardiovascular events. In high-risk hypertensive patients with target organ damage, the structure and function of platelets may be altered. In severely hypertensive patients, the activation of platelets may contribute to the progression of vascular damage. In patients with coronary artery disease, the formation of platelet microaggregates correlates with adverse clinical outcomes.

The effects of hypertension treatment agents on serum lipids have been characterized; however, their antithrombotic properties remain to be investigated. In vitro study has shown that losartan, an angiotensin II type 1 receptor blocker (ARB), can react with thromboxane A2 receptor in human platelets and may possess antithrombotic properties; however, the effects on platelet aggregation among various ARBs have not been fully investigated. In this study, platelet aggregation was assessed by the highly sensitive particle counting method using laser-light scattering to investigate the effects of two types of routinely used ARBs on...
Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Losartan (n=10)</th>
<th>Candesartan (n=6)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72 ± 3</td>
<td>70 ± 5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>136 ± 5</td>
<td>135 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73 ± 3</td>
<td>76 ± 5</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 0.3</td>
<td>26.3 ± 0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>WBC (/mm³)</td>
<td>6430 ± 539</td>
<td>5617 ± 903</td>
<td>n.s.</td>
</tr>
<tr>
<td>Platelet (×10³/mm³)</td>
<td>20.6 ± 2.5</td>
<td>24.7 ± 1.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.8 ± 0.2</td>
<td>5.6 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>195 ± 6</td>
<td>190 ± 16</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>55 ± 4</td>
<td>70 ± 10</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>114 ± 7</td>
<td>100 ± 11</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>131 ± 10</td>
<td>96 ± 17</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

BP: blood pressure; BMI: body mass index; WBC: white blood cell count; Platelet: platelet count; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein. n.s. = not significant. Values are the means ± SE.

Blood Sampling and Platelet Aggregation

Blood samples were collected after an overnight fast in a test tube containing 3.8% sodium citrate. Personnel who performed the laboratory analyses were masked with respect to the clinical data. Samples were centrifuged at 200 g for 15 min and platelet-rich plasma (PRP) was obtained. Samples were then centrifuged at 3,000 g for 10 minutes, and platelet poor plasma (PPP) was obtained. PRP was diluted with PPP to adjust the number of platelets in PRP to 200,000/mm³. Adenosine diphosphate (ADP) was added to PRP at a final concentration of 3 μM. Thromboxane A2 receptor agonist, U46619, was added to PRP at a final concentration of 5 or 10 μM. Platelet aggregation was measured by PA-200 instrument (Kowa, Tokyo, Japan) as previously described. In brief, a laser beam measuring 40 μm in diameter was passed through PRP (300 μL) stirred at 37°C in a cylindrical glass cuvette. The light scattered from the observation volume was detected by a photocell array. Light intensity corresponds to particle size. Data are expressed as the change over time in the number of aggregates (counts/sec) of individual sizes (determined by light intensity expressed as volts). The total light intensities of small, medium and large aggregates were determined. Particles with an intensity of 25 to 400 mV represented small aggregates (9-25 μm, 50-1,100 platelets), those with an intensity of 400-1,000 mV represented medium aggregates (25-50 μm, 1,000-9,000 platelets), and those with an intensity of 1,000-2,048 mV represented large aggregates (50-70 μm, 9,000-25,000 platelets). Changes in signal intensity were recorded at 10-sec intervals for 10 min. Quantitative estimation was performed by determining the area under the curve (AUC) representing the sum of 30 measurements of the light scattering intensity. Spontaneous platelet aggregation was also measured by stirring the PRP without the addition of ADP or U46619.

Statistical Analyses

Results are expressed as the means ± SE. x² analysis was used to determine differences in categorical variables. For continuous variables, statistical analysis was performed by unpaired Student’s t-test. Statistical significance was defined as a p value < 0.05.

Results

Clinical Features of the Subjects Studied

As shown in the Table 1, there were no signifi-
cant differences in age or other clinical features between the two groups. The prevalence of male gender was 50% in the losartan group and 17% in the candesartan group.

Effects on Platelet Aggregation

The detections of small, medium and large platelet aggregates after stimulation with ADP or U46619 were compared. Large platelet aggregation in response to ADP (3 μM) is shown in Fig. 1. Platelet aggregation in patients treated with losartan was less prominent compared to the platelet aggregation in patients treated with candesartan ($p = 0.056$). There were no significant differences in small aggregation (losartan $1.9 \pm 0.3$ vs. candesartan $1.9 \pm 0.3$ ($\times 10^7$) and medium aggregation (losartan $1.2 \pm 0.2$ vs. candesartan $1.5 \pm 0.2$) ($\times 10^7$). Platelet aggregation based on optical density did not differ between the losartan-treated group and the candesartan-treated group (results not shown).

Large platelet aggregation induced by U46619 (10 μM) is shown in Fig. 2. Platelet aggregation in patients treated with losartan was significantly smaller than platelet aggregation in patients treated with candesartan ($p = 0.033$). There were no significant differences in small aggregation (losartan $1.6 \pm 0.2$ vs. candesartan $1.3 \pm 0.1$ ($\times 10^7$) and medium aggregation (losartan $0.9 \pm 0.1$ vs. candesartan $1.1 \pm 0.1$ ($\times 10^7$). At lower concentrations of U46619 (5 μM) there were no statistically significant differences in small aggregation (losartan $1.7 \pm 0.4$ vs. candesartan $1.9 \pm 0.5$) ($\times 10^7$), medium aggregation (losartan $0.5 \pm 0.2$ vs. candesartan $0.7 \pm 0.3$) ($\times 10^7$) and large aggregation (losartan $0.8 \pm 0.3$ vs. candesartan $2.5 \pm 1.6$) ($\times 10^7$). Platelet aggregation based on optical density in response to U46619 (5 and 10 μM) did not differ between the losartan-treated group and the candesartan-treated group (results not shown).

As for spontaneous platelet aggregation, there were no significant differences in small aggregation (losartan $0.6 \pm 0.1$ vs. candesartan $0.7 \pm 0.3$) ($\times 10^7$), medium aggregation (losartan $0.1 \pm 0.1$ vs. candesartan $0.1 \pm 0.1$) ($\times 10^9$) and large aggregation (losartan $0.3 \pm 0.1$ vs. candesartan $0.2 \pm 0.1$) ($\times 10^9$). No correlation was found between platelet aggregation and the control of blood pressure (results not shown).
Discussion

Platelets play important roles in atherothrombosis such as acute myocardial infarction. Platelet aggregation has been conventionally measured by the optical density method and the impedance method. These methods provide crude information about platelet aggregates and do not provide information regarding the number of platelet aggregates of different sizes after stimulation with an agonist. In the light scattering method the intensity of scattered light corresponds to particle size and provides information about various sizes of aggregates formed in the early phase of platelet aggregation. Using the highly-sensitive particle counting method with laser light scattering, this study is the first to quantitate aggregates of different sizes after stimulation with different agonists under treatment with ARBs.

The present study demonstrated significant differences in the effects on platelet aggregation in the native blood of two commonly used types of antihypertensive agents. Candesartan caused significant platelet aggregation as evidenced by laser-light scattering. The other ARB, losartan, caused less platelet aggregation than candesartan, suggesting some differences in the degree of inhibition of platelet aggregation. These results are consistent with the previous study in vitro showing that losartan can react with thromboxane A2 receptor in human platelets. Losartan can react with human platelet thromboxane A2/prostaglandin H2 receptors. Losartan, telmisartan, valsartan and irbesartan can inhibit human platelet aggregation in vitro while candesartan does not possess this property. Metabolites of losartan can decrease human platelet activation mediated by thromboxane A2 receptor.

The therapeutic dose of losartan is known to inhibit platelet aggregation as assessed by conventional aggregometer. Our study confirms this report and extends this observation to non-white Japanese hypertensive patients. Losartan is also reported to reduce markers of platelet activation independent of pressor effects. These results are consistent with the recent results of the LIFE study, which suggested that losartan reduced adverse cardiovascular events compared to atenolol. Taken together, these data indicate that ARBs directly affect platelets, implying that some ARBs likely exert some inhibition on platelet aggregation.

The lack of differences in small and medium aggregates between candesartan and losartan may be due to the relatively high concentrations of ADP and U46619 used in our study. As to the large platelet aggregation in response to lower concentrations of U46619, platelets from the candesartan-treated group tended to be hyperreactive compared with platelets from the losartan-treated group, although statistically not significant. When aspirin is administered orally, it inhibits only the formation of large platelet aggregates. Exposure of platelets to aspirin inhibits their cyclooxygenase activity and decreases the production of thromboxane A2. Therefore, it is likely that losartan mainly affected the formation of U46619-induced large platelet aggregates through its affinity with thromboxane A2 receptor. The conventional optical density method may not have been sensitive to detect the antiaggregatory effect of losartan in our study in contrast to the previous report.

Baseline diastolic blood pressure is associated with the risk of silent brain infarct in Japanese and ARB-based antihypertensive treatment is superior to the conventional treatment for reducing the risk of stroke in Japanese hypertensive patients, especially in patients with a past history of cardiovascular diseases. Our study has shown that some ARBs may inhibit platelet activation in patients with hypertension, independent of blood pressure reduction.

The present study has some limitations. The subject numbers were relatively small and we could not estimate the contribution of factors other than the effects of individual drugs on platelet aggregation. Gender differences in platelet reactivity have been reported. Female platelets were shown to be hyperreactive compared with male platelets in one study but not in others. In this study, women were more prevalent in the candesartan group, which may have affected the results. Further studies with a larger number of patients with a crossover trial protocol are needed to clarify this issue. Although the underlying mechanisms remain unclear, antiaggregatory properties may be independent of angiotensin II type 1 receptor or antihypertensive actions. The novel antiplatelet function can be mediated via its binding to thromboxane A2 receptors on platelets, hence blunting the actions of thromboxane A2 on platelets. This inhibitory effect may be different among ARBs. Losartan has advantageous effects on cerebral circulation. The favorable effects of losartan on the reduction of adverse cardiovascular events among hypertensives may be at least partly mediated by the inhibition of platelet activation.

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